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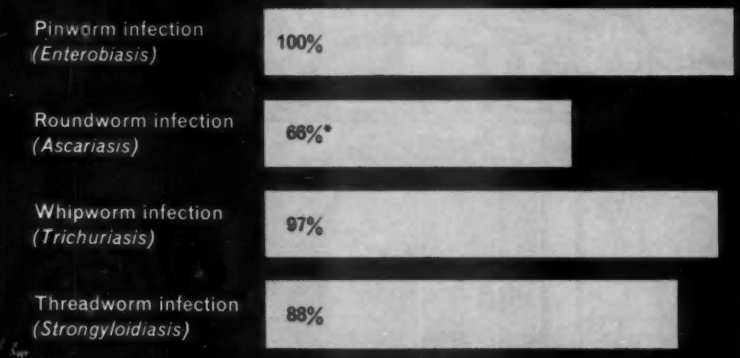

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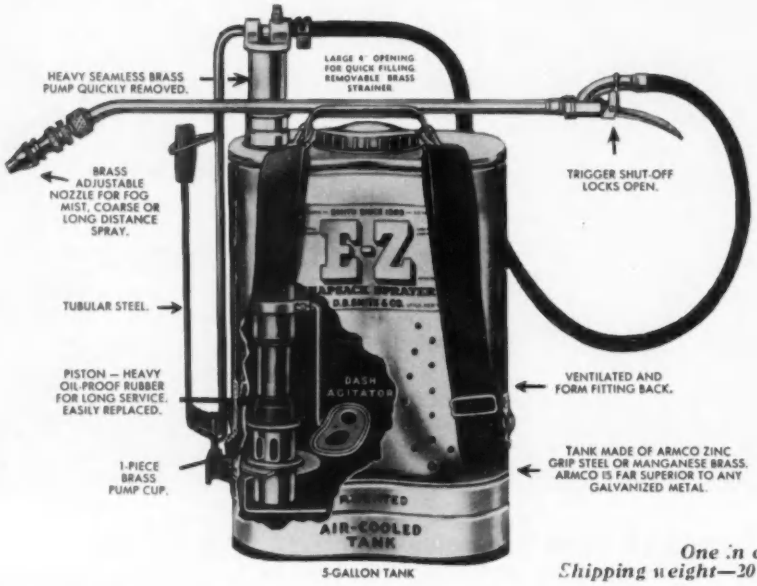
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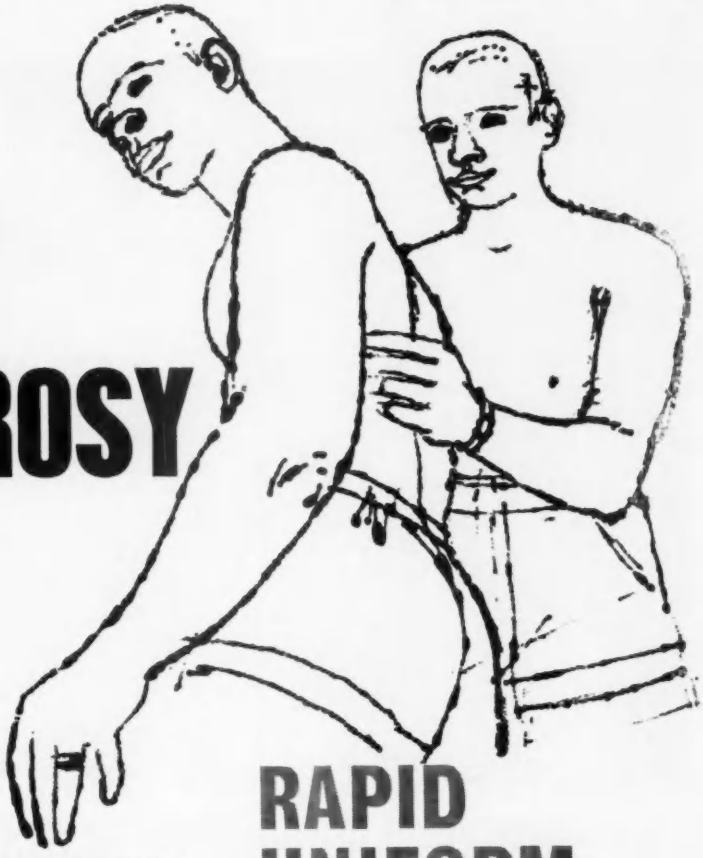

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THE INTERNATIONAL MUNICIPAL WATER SUPPLY PROGRAM: A HEALTH AND ECONOMIC APPRAISAL

JOHN A. LOGAN

Department of Civil Engineering, Northwestern University, Evanston, Illinois

One of the most significant events in the history of sanitary engineering in the twentieth century may well be the decision made by the World Health Organization (WHO) and the International Cooperation Administration (ICA) to cooperate in the promotion of a global program of municipal water supplies.

The World Health Assembly, meeting in Geneva in May, 1959, re-emphasized its previous stand[1] regarding the need and importance of public water supplies, and unanimously approved the Environmental Sanitation Division's plan for a spearhead attack as the first stage in a global program.[2] While WHO has consistently advocated the use of safe water as an important public-health measure, the global plan has two important new features. It recognizes the economic importance of public water supplies and that only part of the cost of a supply is chargeable to health protection. The bulk of the expenditure, to quote the minutes of the Assembly, "is for the provision of a convenience and a commodity"—water supplies should not be promoted as a kind of dole but should be—"operated, managed and maintained on sound business lines."[2] The second new feature of the plan is the shift in emphasis from rural to urban areas. From its inception, WHO has stressed environmental sanitation in rural areas; it is now agreed that sanitation programs can be carried out more economically in areas of population concentration. The plan also takes into account the fact that water supply systems can be established on a paying basis in urban areas, whereas this is difficult, if not impossible, under most rural conditions.

The Public Health Division of ICA is also emphasizing the importance of public water supplies for both health and development. In a recent announcement of policy, the Division stated that

"... a worldwide program for the construction of public water supplies offers a most timely and challenging opportunity for the promotion of public health and for broad social and economic development ... no other single program category offers either the public appeal or the promise of such measurable benefits."[3]

ICA has therefore agreed to collaborate with WHO, the Pan American Health Organization and others in a long-range plan to promote the global program and to assign funds and staff to the project. WHO is proceeding with the establishment of a Water Supply Special Fund and with the preparation of manuals on organization, operation, quality standards, etc. It is hoped that the bulk of the funds needed will be made available for individual projects from international banks, governments and private investors on a business basis. This represents a unique attempt at cooperation between health and financial agencies and should prove to be mutually advantageous.

WATER AND HEALTH

The relationship between water and disease has been recognized for literally thousands of years and pure water is a *sine qua non* of modern healthful living. It is only recently, however, that health workers have recognized that quantity and the ready availability of water are almost as important as quality; if safe water is not readily available in adequate quantities, it encourages carelessness and the use of unprotected supplies; if water is not convenient it is difficult to maintain a satisfactory level of personal cleanliness. The objective, therefore, is not just pure water, but is pure water in ample quantities, piped to individual homes or immediately adjacent thereto.[4]

While water-borne disease is largely a thing of the past in the United States and those countries having modern water supply systems,

"... the widespread inadequacy of domestic water supply is largely responsible for the prevalence of cholera, typhoid and paratyphoid, amoebic dysentery, and the diarrheal diseases, which together are the leading causes of disabling sickness and especially infant deaths in the lesser developed areas throughout the world. ... The

gastro-intestinal diseases are especially prevalent and acute in the lesser developed countries, and account for most of the estimated 500,000,000 persons who suffer from debilitating diseases each year. In many countries, the diarrheal diseases represent the first or second cause of death; on a world-wide basis, they account for about 5 million infant deaths each year."[5]

Of all the basic health measures which can be initiated, the provision of ample supplies of safe water made available to the individual by means of public supplies is generally considered as the one fundamental and most important. In stressing this fact, the 1959 World Health Assembly went on record that public water supply "is the foundation on which rests the health and economic progress of the community."[2]

In many of those parts of the world which now have unsatisfactory water supplies, the economic loss due to sickness and premature death, together with the monetary cost of providing water from vendors or other unprotected sources, would adequately finance new supplies.

WATER AND DEVELOPMENT

At no previous time in the world's history have so many people been concerned with the problems of development, for this is the age in which "human society (has) dared to think of the welfare of the whole human race as a practical objective."[6]

It is trite to say that development is a complicated phenomenon and that there is no single factor on which it depends. There is ample evidence, however, that no program of social and economic development can be permanently successful unless disease can be controlled. As stated by the United Nations:

". . . The advance of any community depends on the extent to which it reduces the burden of ill health which squanders human resources, wastes food in nourishing bacteria and parasites, produces social lethargy, and prevents people and countries from developing their full capacities.[7]

". . . In the underdeveloped areas . . . release of the resources of the countries from the tangled undergrowth of mass disease is a prerequisite of development. Just as the Panama Canal could not be constructed until Gorgas had mastered yellow fever, so the technical resources of these countries cannot be effectively exploited until the conditions of better health are created. . . . Somehow governments (will have) to finance the capital requirements of health."[8]

The progress made in the public-health sciences during the past 60 years has made it clear that the heavy burdens of disease can, in a large measure, be lifted by the application of scientific knowledge. According to no less an authority than Dr. C. E. A. Winslow:

". . . the most dramatic results have been attained in the sphere of environmental sanitation, particularly through improvements in water-supply and waste disposal and in the control of the arthropod vectors of disease."[9]

It can be argued that, it health is fundamental to development, water supply is fundamental to health, and therefore water is a key to development. While community water supplies can be justified on the basis of health alone, they should have a multi-purpose objective, and all of the benefits should be evaluated.

A partial list of the assessable benefits includes:

a. *Health*
   1. Basic physiologic need for water.
   2. Reduction in costs of medical care.
   3. Increased efficiency in human-energy utilization due to reduction in illness and debility.
   4. Reduction in time losses due to illness.
   5. Increased man-power availability.

b. *Economic Development*
   1. Attraction and growth of commerce and industry.
   2. Contributions to production efficiency.
   3. Tourism.
   4. Improved food production.

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g. *Increase in Property Values*

The problem, however, is not just one of whether or not water is important; granting its importance, the question is one of relative importance. With limited capital where does municipal water supply fit into the picture as regards other demands for money: railroads, highways, schools, irrigation, power projects, manufacturing, etc.? This touches directly on the important problems of welfare economics and economic development, subjects which have

been getting increasing attention from economists during recent years.

One of the most significant contributions to this field since World War II, according to *The Economist*, is that presented recently by W. W. Rostow of the Massachusetts Institute of Technology in a series of lectures given during the past year at Cambridge University.[10] Rostow breaks away from the use of the somewhat unsatisfactory terms "underdeveloped" and "developed," and describes social and economic development as an historic process. He classifies countries in accordance with their relative position in economic history as: (1) traditional economics; (2) transitional economics; (3) the take-off period; (4) maturing economies; and (5) period of mass consumption.

In brief, traditional societies are classified as those mainly dependent on agriculture, where food production occupies some 75% of the labor force. They are essentially rural in nature and have a definite ceiling on productivity; they do not make use of modern scientific or technological skills.

The shift from "tradition" to "transition" may come about for a variety of reasons: xenophobia, social decisions, government policy or individual leadership. It demands a recognition of the importance of science, a willingness to take economic risks and an adaptation of the working force.

> ". . . Above all, man must come to regard his physical environment, not as virtually a factor given by nature and providence, but as an ordered world that can be manipulated in ways which yield productive change."[10]

The period of transition is the prelude to "take-off," historically a relatively short period of from 20 to 30 years during which a modern industrial economy is developed. Once a country reaches the take-off stage, development comes quickly and relatively automatically and the "mature economy" stage becomes a foregone conclusion.

The transition period, however, is slow and difficult. During transition, emphasis is usually placed on improved agriculture and the extractive industries and on investment in "social-overhead capital." Social-overhead capital consists primarily of service facilities, transport, water supply, power, irrigation, etc.; governments must usually play an important role in developing this capital and in the preconditioning period in general.

From a public health and water supply point of view, it is the "transitional" and "take-off" periods in which we are primarily interested, for it is during this time that the groundwork is laid for the mature society which is the ultimate objective. Traditional societies are primarily rural and are not in a position to support public works programs; the mature and mass-consumption periods usually have established public water supplies as an integral part of their social organization.

From the point of view of development, the provision of social-overhead capital is not an end in itself, but is rather a basic investment necessary to provide the services needed for such "Directly Productive Activities" (DPA) as mining and manufacturing. These necessary services, according to Hirschman:[11] (1) facilitate or are basic to a wide variety of economic activities; (2) are provided by public agencies or by private agencies subject to some public control; they are usually provided free of charge or at rates regulated by public agencies; (3) cannot be imported; and (4) the investment needed to provide the services is characterized by "lumpiness" (technical indivisibilities) as well as by a high capital-output ratio (provided the output is measurable).

There seems to be no present method of evaluating the relative importance of social-overhead capital as far as development is concerned. Certainly the public services such as law and order, education, public health, transportation, communications, power and water supply are necessary, but as Hirschman points out: "in an underdeveloped country it is often the city with the worst water, power, and housing shortages that is most favored by private investors."[12] Nevertheless, although the provision of public water supply or highways will not bring development by themselves, it is readily granted that Directly Productive Activities cannot progress very far without the support of health, sanitation, communications and transportation. From the point of view of public health, it is sufficient to know that public water supply is acknowledged as a prerequisite to development; unlike certain other services, water has few substitutes and has the distinct advantage of being marketable as a commodity. These "advantages" which have previously been neglected or overlooked by health authorities are obviously major benefits in at-

tempting to promote sanitation and public health on a global basis.

As regards the capital cost of public water supplies as compared to other basic needs, Dr. R. L. Meier has estimated approximately $100 to $300 per capita as compared to an over-all requirement of from $1800 to $3500 per capita for minimum living standards;[13] see Table 1. He further estimates annual costs at approximately $2.00,[14] but this figure appears to be too low and does not represent an adequate return on investment.

In considering the relative importance of water use, there is universal agreement that the highest priority should be for domestic purposes. The President's Water Policy Commission, for example, gives as its first recommendation that "the use of water for domestic purposes should be considered the highest and most universal use by man."[15] There is less agreement on subsequent priorities. S. T. Powell, for example, cites U. S. Department of Commerce figures for 1953 to the effect that the value added by the manufacture of goods averaged $11.70 per thousand gallons of water used, whereas the value of agricultural crops amounted to only $0.075 per thousand gallons, the ratio of manufacturing to agricultural value being 140:1.[16]

Renshaw[17] estimated the relative value of water for various purposes as shown in Table 2. There is no general rule, however, and the use to which water will be placed in any given situation depends largely on local circumstances. As Scheidt points out:

"... Water has many values, depending on location, condition and volume of supply and intended use. In any given location, water is worth what it costs to get it, up to the point where the cost exceeds the price (used here in the broadest social as well as commercial sense) which customers are willing to pay. ... On the question of valuing water on the basis of its productive value, this is only valid if water is the sole remaining requirement for production, as in irrigation."[18]

TABLE 1*

Capital requirements for a minimum standard of living

	Amount per capita
Food	$250–400
Water	100–300
Clothing, shoes and furnishing	50–100
Shelter	400–600
Community facilities	150–300
Transport	150–300
Communications	100–200
Energy sources and transformations	300–800
Other manufacturing and mining	300–500
Total	$1800–3500

* After Meier.[13]

TABLE 2*

Estimated relative value of water for various purposes

Use	Value, $/acre-foot
Domestic	100.19–235.66
Industrial	40.73–163.35
Irrigation	1.67– 27.04
Power	0.71– 5.90
Waste disposal	0.63– 2.56
Inland navigation	0.05– 1.17
Commercial fisheries	0.025– 1.06

* After Renshaw.[17]

WATER AND INDUSTRY

Water is essential to industry as a commodity, aside from any consideration of the health and comfort of employees. In the United States, in 1950, industry used about 120 billion tons of water, more than 50 times the weight of all other industrial materials.[19] The National Association of Manufacturers has stated that "no industry or business can long survive where water is unavailable or inadequate as to the quantity and quality."[20] During the Second World War at least 300 industries had to be abandoned or substantially modified because of inadequate water supply.[19]

There are a number of misconceptions as to the relative importance of municipal water supplies to industry. In the United States, for example, the bulk of water used by industry does not come from public supplies but is produced by the manufacturer, either from wells or from a private surface supply. However, the usual source is the municipal supply, and excluding free water, fire and leakage, commercial and industrial water

use accounts for slightly more than half of all of the water which is sold. According to Seidel and Baumann,[21] of the 497 water supplies serving populations of 10,000 or more which participated in the American Water Works Association (AWWA) survey in 1955, slightly less than 50% of their revenue came from residential sales, about 20% from commercial and about 31% from industrial. The sale of water to industry is therefore an important factor in the operation of municipal supplies.

A relatively few industries use exceptionally large amounts of water and these account for the bulk of industrial water use. The generation of electric power from steam, steel mills, petroleum refining and pulp and paper mills are the biggest users of water. Most of these locate outside of the municipality or depend on their own source of water supply. In a survey conducted by the National Association of Manufacturers, 5.4% of all plants surveyed used 78.2% of all industrial water (those using more than 10,000,000 gallons per day); whereas 67.3% of the plants (those using less than 250,000 gallons of water per day) used only 1.2% of the water.[22] These latter industries included food preparation, beverages, tobacco, chemicals and drugs, lumber and furniture, apparel and textiles, optical, medical and scientific supplies. It is these industries, therefore that are usually dependent on municipal supplies.

These general trends in water use were confirmed by reports presented at an American Association for the Advancement of Science (AAAS) Water for Industry Symposium held in Boston in 1953. It was stated that 5% of the plants used about 80% of the water used by industry, and that this makes water a significant factor in plant location. The large number of smaller plants in a wide variety of industries use municipal water supplies. It was further pointed out that, whereas with the large water users there was great scope for water conservation through reuse and recirculation, the small consumption group could not greatly reduce its consumption. Industries locating in urban areas normally require pure, clean water, and it is to these that municipal supplies must cater.[23] In the United States this amounts to about 55 gallons per capita per day of the total of 150 gallons per capita per day which represents average country-side use (commercial use is about 20 gallons per capita per day).

Municipal water supplies cannot, therefore, be promoted on the basis of their role in the development of heavy industry, but have their primary importance in connection with tourism, commerce and general manufacturing.

The industrial use of water from municipal systems accounts, however, for a significant percentage of the total demand in the United States and this fact should be kept in mind in obtaining support for water-supply programs in the less developed areas.

WATER WORKS ECONOMICS

In assuming that municipal water supplies can be financed on a global basis by the sale of water as a commodity, we are faced with the fact that many municipal water utilities do not now operate at a profit in the United States. This is not to infer that they could not operate profitably, but rather that as a matter of public policy or because of confused accounting systems, water is sold below its cost of production.

Water is the cheapest manufactured commodity in the world. In the United States, for example, water is collected, treated and distributed to individual homes at a cost less than 10 cents per ton. Sanitary engineers take a good deal of pride in this fact, but there is serious doubt as to whether this pride is justified. J. H. Murdoch, in a recent issue of the *Journal of the American Water Works Association*, states emphatically that "we should be ashamed not proud, of too cheap water."[24]

There is no logical argument as to why water should be sold at a cost less than that of production. Although it is in the public interest to encourage the generous use of water for domestic and sanitary purposes, it is also important that people use generous amounts of electricity and that they have adequate amounts of land for housing. Electricity is sold at a profit and land prices are not usually subsidized in the public interest. At present, according to *Fortune* magazine, public water charges are too low to provide the necessary revenue to pay off bonds.[25]

Part of the difficulty lies in inadequate financial and accounting policies. From this point of view there should be no essential difference between private and public water utilities; both should

maintain reserves sufficiently high to "provide adequate service and assure the maintenance, development and perpetuation of the system."[26]

Inadequate rates put the water utility at a distinct disadvantage in terms of service and development; sound long-range plans can be developed only if there is a close and well-understood relationship between engineering and economics. A comparison of electric and water revenues has been made by Learned[27] who surveyed comparative rates of representative utilities; the results indicate that electric utilities pay a considerably higher rate of return on investment than do water supplies (see Table 3).

Another area of misunderstanding is that the use of water does not have to be promoted. There is a widely prevalent concept that water is something which should be furnished without charge rather than a commodity to be purchased. The fact that most municipal water supplies are monopolies is no assurance of their financial success. The liberal use of water is necessary for health, comfort and convenience and a safe, reliable supply is vital to commerce and industry, but the same sound business principles must be used in the promotion and sale of water as in the sale of gas or electricity.

The history of water supply in the United States indicates that municipal supplies received wide public acceptance only after they achieved adequate reliability and a high standard of quality. Prior to 1900 most public supplies had to compete with private wells and cisterns. In Butler, Pennsylvania, for example, 20 years following the opening of the water system in 1877, there were only 500 service taps for a population of 9000. In Washington, Pennsylvania, the water utility was opened in 1887; by 1897 only 460 service taps were in use for a population of 14,000 (1:30); by 1910, 3000 taps for 20,000 (1:6.6) and by 1954 there were 18,000 service taps for 69,000 people, a ratio of 1:3.9.[24]

TABLE 3*

Investment and revenue of electric and water utilities

Type	Investment dollars per dollar of annual revenue	Average revenue ($)		
		Per residential customer	Per commercial customer	Over-all
Electric utilities.....	4.21	74.83	756.93	168.50
20 private water utilities.....	6.34			48.68
1 large municipal water utility.....	10.00	44.90		68.63

* After Learned.[27]

These examples are not unusual. Historically, public water supplies have had to compete with individual supplies which were sometimes more reliable and cheaper, although usually not safer than the public supply. Many private utilities failed before they could get enough customers to reach the break-even point. To be used at all, prices had to be low; our present pricing is in part, at least, a carry-over from this attitude.

Rates and rate structures have been the subject of intensive study during recent years by the AWWA and other similar groups and a considerable volume of literature is available on this topic (see references 26, 28 and 29). The problem of financing under U. S. conditions has also been widely discussed; although there is some disagreement as to the ideal method, Wolman probably expresses a majority opinion in his summary of the subject in the December 1956 issue of the *Journal of the American Water Works Association*.[30] He advocates a broad base of support, an *ad valorem* tax for large mains for future service, front-foot assessment for individual homes and water consumption payment for actual water use. Wolman does not believe that water rates should attempt to cover all the costs of a water utility, nor does he advocate revenue bonds, which, if used without general tax support, assume that the water user has the sole responsibility for payment.

ORGANIZING FOR THE GLOBAL PROGRAM

The developing areas of the world need municipal water supplies as an essential to health, comfort and economic progress. Modern engineering methods make it possible to provide adequate quantities of pure water to individual homes in most of the urban areas of the world at a very low price. Water utilities are reliable and long lived; they can be readily installed and operated; their cost in many cases would not exceed what people now pay for inferior and unsafe supplies.

A unique opportunity exists to meet an im-

portant need by the application of available technological knowledge. Water is urgently needed; the important factor missing is the organization which will make it possible to establish water authorities, finance and operate them.

Although there is still important engineering and developmental work to do on the use of new materials, design and quality standards and the optimization of treatment plants and service lines, these are relatively straightforward problems and work on them is already being undertaken by ICA, the Environmental Sanitation Division of the World Health Organization and others. Additional research is needed on problems such as the benefits of water to various industries and to commerce, the monetary value of water to health and the value of a municipal supply to public cleansing, fire protection, personal convenience, etc. Water has so many inherent advantages, however, that its relative values become of secondary importance.

The consideration of water supply development from an economic rather than a social point of view poses new problems to the public health profession. A number of the problems have been brought out in this paper. Their solution will require the combined efforts of engineers, doctors, economists and administrators. Fortunately none of the problems is critical and there is no reason why the global program cannot move boldly forward on the basis of existing knowledge, while, in the meantime, every effort is made to improve both techniques and organization.

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SEASONAL VARIATION IN VENOM OF BLACK WIDOW SPIDERS

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Contrary to popular belief, seasonal variation in toxicity of venom samples obtained from snakes, spiders, and scorpions has not previously been demonstrated. It is well known that variation of unknown etiology in amounts and toxicity of venom yields from individual animals occurs without regard to season. Examples of such variation in snake venom are cited by Minton[1] and Christensen.[2] Schöttler,[3] who noted qualitative and quantitative variations among venom samples from captive scorpions, speculated upon a possible seasonal linkage but was unable to conduct his observations over a sufficient period of time to confirm his suspicions. D'Amour *et al.*[4] found differences in toxicity of venom samples from black widow spiders captured in Colorado and California in July and October, respectively, and also raised the question of possible seasonal influence. Bücherl,[5] working with *Phoneutria nigriventer* (Keyserling, 1891), a South American spider, found no difference in toxicity of venom extracted in winter and summer months. He did find that the amount of venom obtained by allowing spiders to bite through a rubber sheet was greater in warm weather. Wiener[6] tested effects of the venom of the Australian red-back spider, *Latrodectus hasseltii* (Thorell, 1870), with white mice and found that the temperatures at which the experimental mice were held had a marked effect on results of venom potency tests.

In this paper, evidence is presented of seasonal variation in toxicity for white mice of intraperitoneal injections of triturated cephalothoraces of freshly caught black widow spiders from Camp Bullis, Texas. The seven potency tests which serve as the basis for this report were conducted in November, 1958, and during February, April, May, August, and November, 1959.

MATERIALS AND METHODS

Adult female black widow spiders, *Lactrodectus mactans* (Fabr., 1775), used in the potency tests were found beneath rocks, logs, and boards. Specimens were available throughout the year. with a marked decrease in the population during July and August. When a sufficient number of spiders for a potency test was not found on one day, all captured specimens were left caged under natural conditions in the field until an adequate number had been obtained. In most instances spiders were not kept in the laboratory more than 48 hours before use. Immediately before the potency tests, spiders were anesthetized, abdomens and legs removed, and cephalothoraces triturated in saline in a mortar. The stock solution of triturated cephalothoraces in saline was then strained through gauze to remove sclerotized particles. Each dilution from the stock solution was prepared immediately before use. Twenty-eight day old white mice weighing 14 to 18 grams and obtained from a single source were used as test animals.

Injections of cephalothoracic contents in saline of varying concentrations in a constant volume of 0.25 ml were administered intraperitoneally in the lower, left quadrant of the abdomen with a 23-gauge needle. These injections were given by the same individual throughout the testing program. After receiving injections, mice were placed in animal jars with ample food and water and observed for a 24-hour period. Death within 24 hours was the criterion for toxicity. Since each spider cephalothorax contains two venom glands, injections and results are interpreted in terms of fraction of a gland contained in the inoculum.

In all calculations of the LD_{50} the method of Reed and Muench[10] was employed. Estimates of the standard error of the log of the LD_{50}'s were computed by the formula of Pizzi.[11]

RESULTS AND DISCUSSION

As indicated in Table 1, highest toxicity of injected black widow spider cephalothoraces for white mice occurred in November of 1958 and 1959. Lowest toxicity occurred in April and May, 1959. The basis for this variation is not known. Preliminary studies conducted in this laboratory indicate that temperature alone is not the basic

TABLE 1

*Toxicity for white mice of intraperitoneal injections of triturated cephalothoraces of black widow spiders from Camp Bullis, Texas**

Test Date	Dilutions		Number of mice		LD	Standard error of log of LD	95% confidence limits of LD
	No.	Range	Per dilution	Total			
21 Nov 58	8	.11–.75	10	80	.33	.067	.24–.45
17 Feb 59	6	.10–.83	10	60	.43	.073	.31–.60
25 Apr 59	6	1.50–4.00	10	60	3.29	.098	2.09–5.16
4 May 59	5	1.50–6.00	10	50	2.45	.064	1.82–3.29
31 Aug 59	2	.76–1.32	10	20	ca 1.00†		
9 Nov 59	4	.67–2.25	20	80	<.67‡		
23 Nov 59	6	.15–1.00	10	60	.23	.071	.17–.32

* Each injection in a constant volume of 0.25 ml, but with varying fractions of cephalothorax in saline.

† Insufficient numbers of spiders for accurate estimation.

‡ Deaths at all dilutions exceeded 50%.

factor involved. Spiders were held at various temperatures from 35°F up to room temperature, and LD_{50} values of triturated cephalothoraces for white mice were determined. When this was done with spiders collected at the same time and place, there were no marked differences in LD_{50} values obtained with specimens which had been held at room temperature, and those held at lower temperatures.

D'Amour *et al.*[4] expressed the opinion that use of macerated cephalothoraces, rather than dissected whole glands, for venom potency tests would give wide variation in results. This is undoubtedly true unless care is taken in trituration of cephalothoraces. If sufficient care is taken, consistent results can be obtained by this method. Such consistency is shown in reports by Sampayo,[7] Keegan,[8] and Stanic.[9] These workers not only obtained consistent results, but LD_{50} values, expressed in fraction of a gland or cephalothorax injected, which agree with one another, and as nearly as can be determined, compare well with the results of D'Amour and his co-workers, who used white rats as test animals.

The two obvious disadvantages in use of triturated cephalothorax are the lack of certainty as to the actual amount of venom injected and the possible interference of body fluids and tissue juices with test results. The latter of these does not appear to have been of great importance in tests such as those reported in this paper, but, as Schöttler[3] points out, could conceivably introduce antigenic substances which could interfere with antivenin production programs.

SUMMARY

Seasonal variation in toxicity for white mice of intraperitoneal injections of triturated cephalothoraces of freshly caught black widow spiders, *Latrodectus mactans* (Fabr., 1775), was demonstrated. Spiders used in these tests were collected at Camp Bullis, Texas. Twenty-eight day old white mice from a single source were used as test animals. Intraperitoneal injections were of varying strength with respect to fraction of a cephalothorax included in a constant volume of 0.25 ml of saline solution. LD_{50}'s were expressed as fractions of a venom gland contained in the injection. Highest toxicity, as indicated by LD_{50}'s of .33 and .23, occurred in November of 1958 and 1959. Lowest toxicity, as shown by LD_{50}'s of 3.29 and 2.45, occurred in April and May, 1959.

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CHEMORECEPTION IN THE SNAIL *AUSTRALORBIS GLABRATUS**

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Studies on chemoreception in gastropod molluscs have been limited to investigations of marine prosobranchs, and apparently no information is available on chemoreception in aquatic pulmonates (Basommatophora). Chemoreception in gastropods is generally attributed to an organ called the osphradium. This is based largely upon evidence derived from anatomical studies,[1] or from circumstantial evidence and uncontrolled experiments.[2,3] Copeland[4] provided limited experimental evidence that the osphradium of some carnivorous prosobranchs may function as a chemoreceptor, and Hulbert and Yonge[5] concluded that the main function of the osphradium in certain prosobranchs was to test the amount of sediment carried into the mantle cavity by water currents.

Observations in our laboratory have shown that a complex alginate-based snail food[6] (as modified by Moore *et al.*)[7] apparently attracts such aquatic snails as *Australorbis glabratus*, *Biomphalaria pfeifferi*, and *Helisoma anceps*. It has been noted that snails regularly leave lettuce or watercress and congregate upon the alginate-based food soon after it is placed in an aquarium.[8] Preliminary experiments with *A. glabratus* showed that within 6 to 8 hours after introduction 60 to 80% of the snails in an aquarium could be found either feeding on or in the vicinity of a strip of the alginate food. A series of experiments was devised to determine whether the alginate-based food, as well as other substances, actually served as attractants for snails. Experiments to elucidate the function of the osphradium with respect to chemoreception were also undertaken.

MATERIALS AND METHODS

General

Snails of the PR-1 strain[9] of *A. glabratus* were used in all experiments. This strain is of Puerto Rican origin and has been maintained for 6 years as previously described.[8]

The reactions of test snails to various baits were determined by observing the behavior of a series of individuals in a "Y"-shaped maze constructed of clear plastic (Figure 1) with a capacity of 9.5 ml. All experiments were conducted with snails 3.0 to 6.0 mm in diameter, since the depth of this maze precluded the use of larger snails.

Each bait was tested in 24 individual trials ("bait series") conducted as follows. Snails of a suitable size were selected from stock *A. glabratus* and starved for periods of 3 to 6 hours before use. The maze was placed on a background of glossy black paper and filled with aquarium water at room temperature (25 ± 1°C). A test snail was placed in the starting well for a 2-minute conditioning period prior to the introduction of the bait into 1 of the 2 test wells. Every trial was timed from the introduction of the bait and was terminated when the snail entered 1 of the 2 test wells, or at the end of 1 hour. If a snail failed to reach a well during the allotted period, it was discarded and another snail substituted. Three was the maximum number of snails which had to be discarded in any one trial series, but in most series it was not necessary to replace any. A new snail and a fresh sample of bait were used in each trial, and alternate test wells were baited in successive trials. The maze was emptied between trials, washed in hot tap water, and dried with paper toweling. A 60-watt bulb was placed 18 inches above the maze in preliminary experiments; however, since trials with the same bait yielded similar results under ordinary ceiling-level fluorescent lights, the latter method was used routinely.

Each bait series of 24 individual trials was treated statistically as a unit. The Chi-square Test with Yates' adjustment for correcting small samples was used in the statistical evaluation. Chi-square values greater than 6.635 ($P = 0.01$) were considered significant. In order to characterize the results of tests a null hypothesis was adopted, *i.e.*, snails would react in a

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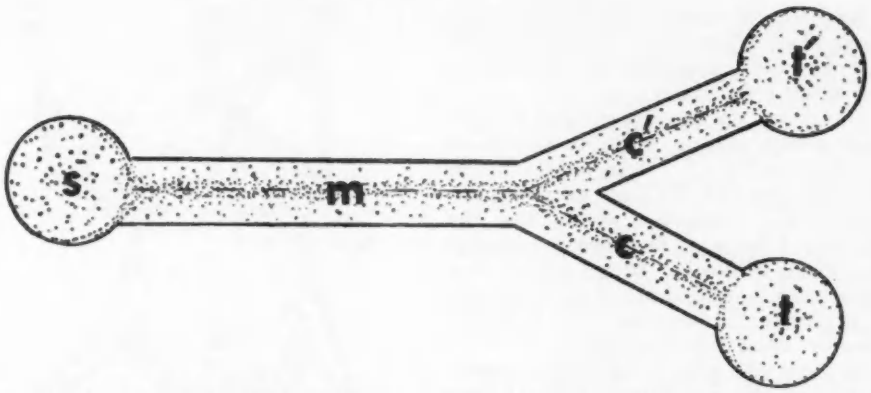

FIG. 1. Diagrammatic sketch of "Y"-shaped maze viewed from above. Dimensions: starting well(s) and test wells (t & t'), diam 20 mm, depth 6 mm; main channel (m), 60 x 10 x 6 mm; side channels (c & c'), 35 x 10 x 6 mm. The sides of all channels were inclined at an angle of 45°.

random manner with respect to entering one or the other of the test wells regardless of the bait involved. However, if the number of individual snails entering the baited well was significantly greater than could be expected by random chance, the bait was considered "attractive." A bait was considered "repellent" if a significant number of snails avoided the baited well. Baits which resulted in a random distribution of test snails were considered "non-reactive." The latter term is deliberately noncommittal since either very rapid or very slow diffusion from a test bait might result in random responses on the part of the experimental snails.

Preparation of Baits

Thirteen baits were tested and in addition a feeding snail was placed in the test well and used as bait. A description of the baits, the method of preparation, and the conditions of use follow. Unless otherwise indicated, baits were used within 24 hours of preparation.

Filter paper discs. Discs 6 mm in diameter were punched from sheets of Whatman #3 filter paper. These discs served as vehicles for certain of the baits.

Human feces. Filter paper discs were coated with feces from a healthy adult. Coated discs were introduced as bait within an hour after the feces had been passed.

Human urine. Filter paper discs were soaked for 1 hour in urine freshly passed by a healthy adult. The discs were removed from the urine, placed on glass plates and permitted to dry for about 1 hour at room temperature (25 ± 1°C) before use.

Romaine lettuce (Lactuca saiva, *var.* longiafolia). Commercially grown romaine lettuce supports both growth and reproduction of *A. glabratus* in our laboratory. Pieces of leaf (5 mm²) were freshly cut and introduced at once into test wells.

Watercress (Nasturtium officinale). Commercially grown watercress is regularly used as the main aquatic plant for stock colonies of *A. glabratus* since it serves both as a source of food and as a site for oviposition.[8] Pieces of watercress leaf (5 mm²) used as bait were freshly cut from plants growing in stock aquaria.

Ground watercress. Watercress leaves were ground in a mortar and 0.3 to 0.5 g of the freshly ground material was immediately introduced into a test well.

Chlorophyll extract of watercress. Twenty-five grams of watercress leaf was immersed in 50 ml of boiling absolute alcohol for 5 minutes. The mixture was then stored for 24 hours at 5°C, after which filter paper discs were immersed in the supernatant liquid for 1 hour, and the discs then dried on glass plates at room temperature.

Alginate-based snail food. This food was prepared from the formulation of Moore *et al.*[7] It was formed into filamentous strands by injecting the liquid alginate-food mixture into the calcium chloride solution. The strands were washed and stored in distilled water at 5°C. Approximately 1 g (wet weight) of this food was used as bait per trial.

Components of the alginate-based food. According to Moore's formulation,[7] the alginate-based food consists of 5 components, each of which was individually tested as bait.

(a) Calcium alginate. One gram of standard grade sodium alginate (Algin Corporation of America, New York, N. Y.) was added to 100 ml of distilled water (at 70°C), and stirred continuously until a homogeneous viscous mass was produced. Filter paper discs were immersed in the alginate solution for 10 minutes and then transferred into a 2% solution of calcium chloride for 10 minutes. The discs were washed in distilled water and stored in distilled water at 5°C.

(b) Dried skim milk. A water soluble powder of skim milk was used (Evangeline Brand, First National Stores). Filter paper discs were immersed for 10 minutes in a 0.5% solution in distilled water. The discs were allowed to dry on glass plates at room temperature and stored dry at 5°C.

(c) Cerophyl (Cerophyl Laboratories, Inc., Kansas City, Mo.). This is a dehydrated form of powdered cereal grass leaves. Cerophyl was mixed with distilled water to form a thick paste and about 0.3 g was added directly as bait.

(d) Glandex (Burnett's Foods, Inc., Newark, N. J.). This is a commercial brand of fish food in the form of a fine powder. Baits were prepared by making a paste with distilled water and adding 0.3 g of the paste directly to the test well.

(e) Wheat germ (Kretchmer Wheat Germ Corporation, Carrollton, Mich.). This product was obtained in the form of small flakes which were mixed with distilled water to form a paste; 0.3 g of paste was added directly as bait.

Feeding snails. As an additional type of bait, small *A. glabratus* (shell diam 3–5 mm) were placed in a test well and permitted to feed on a piece of watercress leaf (5 mm^2). A barrier of perforated polyvinyl plastic prevented the excape of the feeding snail, but permitted diffusion of substances through the maze.

Technique for the Destruction of the Osphradium

The osphradium in *A. glabratus* and other pulmonate snails is extra-pallial (Figure 2), differing in this respect from the prosobranch molluscs. Furthermore, the osphradium in *A. glabratus* is a ciliated pit rather than the complex filamentous structure observed in prosobranchs. Dissection discloses a large ganglion which lies below the osphradium and anastomoses with it (Figure 3). The ganglion is joined by a large nerve which passes through the tissues on the left side of the neck, thence beneath the preputium to the left parietal ganglion from which it originates. A smaller nerve originates from the osphradial ganglion and innervates the muscles of the pneumostome. There also appears to be a nerve net surrounding the osphradial pit. Histological studies confirmed the detailed investigations of Pan,[10] and verified the presence of ciliated, sensory, columnar epithelium lining the osphradial pit.

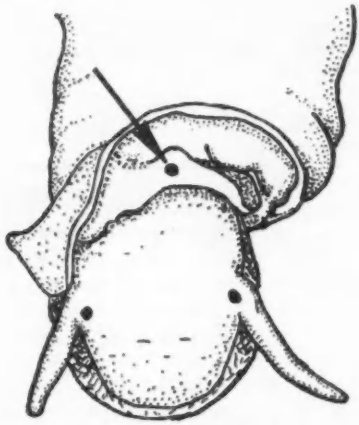

FIG. 2. Diagrammatic sketch of the cephalopedal region of the snail *Australorbis glabratus*. The mantle collar has been partially reflected to show the extra-pallial osphradial pit (arrow).

The techniques used in the destruction of the osphradium were as follows. Snails were first extended and immobilized by means of urethane and a micro-retractor in accordance with a technique previously described.[11] The osphradium was then exposed by reflecting the mantle collar with forceps. The osphradium and/or its ganglion were then destroyed with a micro-cautery similar to one described by Noyes.[12]

Gross and histologic examination of five cauterized snails demonstrated that the osphradium and/or the osphradial ganglion of these snails were destroyed and replaced by "scar" tissue composed of fibroblasts and dense connective tissue that possessed an increased amount of pigmentation. All procedures were done under low magnification (13 ×). Postoperative recovery rates were high (80–90%). Snails were used in

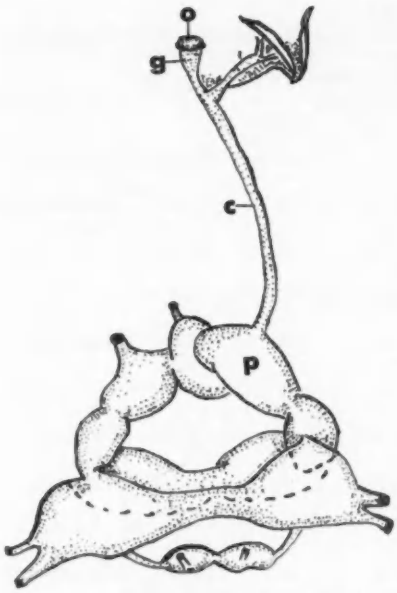

FIG. 3. Diagrammatic sketch illustrating the relationship of the osphradium and the osphradial ganglion to the circumpharyngeal ganglionic complex: o, osphradium; g, osphradial ganglion; c, parietal-osphradial commissure; p, parietal ganglion.

experiments after being held in aerated aquaria for 48 hours. To provide sham-operated controls, normal snails were immobilized and extended as described above, but were then cauterized on the foot or the mantle collar; these were also held 48 hours before use.

The surgical procedures required the use of snails 15 to 18 mm in diameter. Since the plastic maze described earlier was too small for bait tests with snails of this size, a larger "Y"-shaped maze was constructed from plaster of Paris waterproofed with spar varnish (main channel, 14.5 x 4.5 x 2.5 cm; arm channels, each 10.0 x 2.5 x 2.5 cm; volume, 225 ml).

EXPERIMENTAL RESULTS

Bait Studies

Before individual baits could be tested it was first necessary to determine whether snails themselves, or some factor in the construction of the plastic maze, would predispose to experimental bias. Furthermore, since filter paper discs were used as vehicles for the introduction of certain baits, it was also necessary to determine if the discs per se would influence snail movements. Data obtained from 2 series of snails tested in the unbaited maze, and from 1 series of snails tested in the maze "baited" with filter paper discs, showed a random distribution with respect to the selection by snails of one or the other of the test wells. It followed from these experiments that any significant deviation from a random distribution could be attributed to the particular bait under test. These and all other experimental results in this section are summarized in Table 1.

It is evident from the results of 3 series of tests that the complete alginate-based food was highly attractive to *A. glabratus*. Further studies on the individual components of this food then revealed that snails were attacted only by the wheat germ component. All other ingredients of the alginate-based food (calcium alginate, skim milk, Cerophyl, Glandex) were tested and classified as non-reactive, as were each of the following baits: human feces, human urine, watercress leaf, a chlorophyll extract of watercress leaf, and romaine lettuce. Feeding snails were also classified as non-reactive baits since test snails were not attracted to them.

One bait, ground watercress, proved to have a repellent effect on *A. glabratus*. In the presence of this bait a large proportion of test snails crawled out of the maze entirely, while some of the remaining snails retracted into their shells and failed to leave the starting well. The behavior of the snails seemed clearly to indicate an avoidance reaction to a noxious substance, but the basis for this reaction was not elucidated.

Studies on the Function of the Osphradium

Preliminary tests were done to determine if adult snails placed in the plaster of Paris maze would behave in a random manner in the absence of bait, and in a selective manner in the presence of the alginate-based food. These experiments yielded results similar to those obtained earlier with the small snails. Thus, 2 series, each of 24 large snails, tested in the unbaited maze demonstrated random behavior patterns, whereas in 2 series tested in the presence of the alginate-based food, 21 of 24 and 19 of 24 snails entered the baited well ($\chi^2 = 12.042$ and 7.042, respectively). In the latter experiments, and in those described

TABLE 1

Summary of experimental results on the attractiveness of selected baits to Australorbis glabratus

Baits	Mean shell diam & range (mm)	No. snails entering*		x^2†	Bait classification
		Baited well	Empty well		
None					
Series 1	4.6 (3.6–5.2)	13	11	<1.000	Random distribution
Series 2	4.4 (4.1–5.0)	10	14	<1.000	
Filter paper discs					
Series 1	4.6 (4.0–5.0)	13	11	<1.000	Non-reactive
Human feces					
Series 1	4.6 (3.1–5.3)	13	11	<1.000	Non-reactive
Series 2	3.4 (3.0–4.1)	16	8	2.042	
Human urine					
Series 1	4.8 (3.4–6.0)	14	10	<1.000	Non-reactive
Series 2	4.7 (3.4–5.8)	12	12	0.000	
Romaine lettuce					
Series 1	4.5 (3.6–5.5)	14	10	<1.000	Non-reactive
Watercress leaf					
Series 1	5.5 (4.9–6.0)	9	15	1.042	Non-reactive
Series 2	4.6 (3.1–5.4)	13	11	<1.000	
Ground watercress leaf					
Series 1	3.8 (3.0–4.5)	4	20‡	9.375	Repellent
Series 2	5.0 (4.2–5.8)	3	21§	12.042	
Series 3	4.7 (4.2–5.6)	4	20‖	9.375	
Chlorophyll extract (watercress leaf)					
Series 1	5.6 (4.4–5.9)	13	11	<1.000	Non-reactive
Alginate-based food					
Series 1	4.5 (3.8–5.2)	21	3	12.042	Attractive
Series 2	5.5 (4.2–6.0)	22	2	15.042	
Series 3	5.2 (4.5–5.6)	20	4	9.375	

TABLE 1—*Continued*

Baits	Mean shell diam & range (mm)	No. snails entering*		x^2†	Bait classification
		Baited well	Empty well		
Components of alginate-based food					
Calcium alginate					
Series 1	4.7 (3.4–5.5)	11	13	<1.000	Non-reactive
Skim milk					
Series 1	4.5 (4.0–5.0)	14	10	<1.000	Non-reactive
Cerophyl					
Series 1	4.5 (4.1–5.4)	16	8	2.042	Non-reactive
Glandex					
Series 1	5.1 (3.9–5.7)	17	7	3.375	Non-reactive
Wheat germ					
Series 1	4.5 (4.1–5.0)	21	3	12.042	Attractive
Series 2	4.9 (4.0–6.0)	20	4	9.375	Attractive
Series 3	5.2 (4.5–6.0)	21	3	12.042	Attractive
Feeding snails					
Series 1	5.2 (4.7–5.8)	15	9	1.042	Non-reactive

* In 7 of 25 bait series it was necessary to discard and replace from 1 to 3 snails for failing to select one or the other of the test wells in the allotted time.

† Values corrected for small samples. Values greater than 6.635 ($P = 0.01$) were considered significant.

‡ This value includes 7 snails which crawled out of the maze and 5 snails which withdrew into their shells and remained in the starting well.

§ This value includes 14 snails which crawled out of the maze.

‖ This value includes 5 snails which crawled out of the maze and 4 snails which withdrew into their shells and remained in the starting well.

below, 5 g (wet weight) of the complete alginate-based food served as bait in each experimental trial.

Two experiments were devised to evaluate the role of the osphradium in enabling *A. glabratus* to find an attractive bait. In one experiment, three groups of snails of similar mean shell diameter (15.9–16.1 mm) were selected from the stock colony and treated as follows. In the first group, the osphradium and/or the osphradial ganglion were destroyed by cauterization; snails in the second group were cauterized on the foot or mantle collar (sham-operated controls); a third group was left untreated (normal controls). All snails in each group were then tested in the maze baited with alginate-based food. Since limitations of time imposed by surgical procedures restricted the number of snails per series, the data for all series in each treatment group were combined for statistical evaluation. The results (Table 2) clearly show that highly significant proportions of the normal controls and of the sham-operated controls selected the baited well, whereas under similar test conditions the osphradium-cauterized snails behaved in a random manner. These data indicate that the osphradium functions as a chemoreceptor and that the ability of the snails to locate an attractive bait is, at least in part, dependent upon the integrity of this organ.

A variation of the preceding experiment was conducted to obtain additional corroborative evidence. Five snails of similar mean shell diameter (15.1–15.4 mm) were tested (24 trials each)

TABLE 2

Comparison of the behavior of normal snails, sham-operated snails, and osphradium-cauterized snails in a maze baited with alginate-based food

Series	Condition of snails					
	Osphradium cauterized		Sham-operated controls		Unoperated controls	
	Snails tested		Snails tested		Snails tested	
	Total no.	No. located baits	Total no.	No. located baits	Total no.	No. located baits
1	7	3	10	8	5	4
2	9	3	10	7	13	10
3	5	3	8	5	11	8
4	9	4	9	8	13	9
5	6	3	8	5	15	12
6	10	4	10	7	6	5
7	10	6	10	8		
8	9	3	10	6		
Totals	65	29	75	54	63	48
χ^2	0.754		14.520		17.286	

TABLE 3

Individual behavior of 5 snails in a baited maze before and after the destruction of the osphradium

Snail no.	Pre-operatively		Post-operatively	
	Number of trials bait selected†	Chi-square*	Number of trials bait selected	Chi-square*
1	20/24	9.375	16/24	2.042
2	21/24	12.042	13/24	<0.100
3	21/24	12.042	14/24	0.375
4	20/24	9.375	13/24	<0.100
5	22/24	15.042	10/24	<0.100

* Chi-square values corrected for small samples. Values greater than 6.635 (P = 0.01) were considered significant.

† Denominator indicates total number of trials. From 0 to 2 repeat trials were necessary in any one trial series for snails which failed to select one or the other of the test wells in the allotted time.

in the maze baited with alginate-based food. The osphradial ganglion of each snail was then destroyed by cauterization and the snails retested (24 trials each) in a similarly baited maze. The data (Table 3) for all trials done pre-operatively show that normal snails readily reached the bait; however, when the same snails were retested in the baited maze after the osphradia had been destroyed, only random behavior was evident. These observations serve to confirm the results of the previous experiment in demonstrating the function of the osphradium in chemoreception.

DISCUSSION

Although laboratory data cannot be safely transposed to field situations, the present study suggests the possibility of the use of baited traps in the sampling, and, perhaps, in the control of snail populations. The incorporation of a suitable oral or contact poison into an attractive bait may prove to be an efficient way to kill vector snails under conditions where molluscicides are difficult to use.

Traps for the control or sampling of snail populations have been made from such diverse materials as screening, cans, or palm fronds. These function by mechanical filtration of snails from water currents, although several reports imply some attraction of snails to the palm frond type of trap.[13-16] However, Witenberg and Saliternik[17] found that traps deliberately baited with various plant leaves, or with Standen's alginate-based food, failed to attract *Bulinus truncatus* under field conditions (Yarkon River, Israel), and concluded "... that the use of traps for determining the presence or density of snail populations is impracticable in local conditions." Critical evaluation of this report is not possible since insufficient data are presented. However, it is of interest that preliminary trials conducted in the small "Y" maze with an Egyptian strain of *B. truncatus* indicate that this snail behaves randomly in the presence of the alginate-based food. Different attractants may have to be sought for different types of related snails.

It is of some interest that baits prepared from human feces and urine were, under present experimental conditions, not attractive to *A. glabratus*. These observations are in contrast to previous reports which imply that many snail species "prefer" feces as food,[18, 19] and that *A. glabratus* and other Planorbidae are attracted to feces.[20] Unpublished evidence obtained by the present author indicates that snails will actively feed upon feces, and that urine may even stimulate growth and reproduction; however, so far there is no evidence that these substances actively attract snails.

In general terms, the effectiveness of any given bait would seem to depend, in part, on its physical and chemical properties, and, in part, on the physical and chemical characteristics of the aquatic habitat. However, it is obvious that an effective attractant must first be able to stimulate the chemoreceptor(s) of the snails and cause them to seek the bait actively. In this regard, it has been demonstrated experimentally that snails are capable of an active "seeking response" in the presence of certain substances, and that the osphradium is of major importance in this process.

SUMMARY

Fourteen baits were tested in a "Y"-shaped maze for their attractiveness to the snail *Australorbis glabratus*. Wheat germ and an alginate-based snail food containing wheat germ were the only baits which attracted snails. One bait, ground watercress leaf, acted as a repellent. All other baits were found to be non-reactive. Human feces and urine did not appear to attract this snail.

Experiments also demonstrated that the osphradial organ is a chemoreceptor in *A. glabratus* and functions in the detection of attractive substances.

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THE COMPARATIVE EFFICACY OF BEPHENIUM HYDROXYNAPHTHOATE AND TETRACHLOROETHYLENE AGAINST HOOKWORM AND OTHER PARASITES OF MAN

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Recently we reported that bephenium chloride, a member of a series of new quaternary ammonium compounds, showed promising activity against hookworm infections (*Necator americanus*) in mental patients.[1] About the same time Goodwin *et al.* using different bephenium salts reported similar results from Ceylon.[2] They found the hydroxynaphthoate salt to be more suitable than the bromide or embonate salts. In both of these studies the reduction in egg density was far greater than the cure rate.

The above studies, together with that of Nagaty and Rifaat,[3] indicated that very few side effects resulted from the use of the bephenium salts. Rogers reported that bephenium is poorly absorbed from the gut in man.[4]

The present study was undertaken to determine the efficacy of the hydroxynaphthoate salt of bephenium against worm infections, usually of high intensity, in mental patients. Tetrachloroethylene, a commonly used anthelmintic, was used for comparison.

MATERIALS AND METHODS

The patients were white and Negro female residents of a mental hospital.

The bephenium was given usually 1 to 2 hours after meals. The single daily doses were given after the morning meal, twice daily doses after the morning and evening meals, and thrice daily doses after each of the three meals. There were no pretreatment or post-treatment procedures, such as purges or fasting. The drug was given in a fine granular form (Alcopara®)‡ suspended in fruit juice, milk, or, occasionally, in water. The dosages of bephenium hydroxynaphthoate are shown in terms of grams of the base.

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‡ Burroughs-Wellcome furnished the bephenium hydroxynaphthoate salt under the trade names of "Alcopar" and "Alcopara."

The tetrachloroethylene was given in a single dose of 3 or 4 ml. All patients receiving this drug had the usual light meal the evening before treatment. Half of those who were to receive 3 ml had a purge later in the evening and also 2 hours after the dose of tetrachloroethylene. The drug was given about nine o'clock the following morning after about 16 hours of fasting.

The density of the worm eggs in the feces was determined by the Stoll method corrected to formed stool basis. Two daily determinations were done before the drug was given. Usually 3 weeks after the treatment two stool specimens were examined by a zinc sulfate flotation method and if eggs were found the density was measured by the Stoll method.

The patients were supervised carefully during and after the administration of the drugs. Any side effects were immediately called to the attention of the ward physician. Such reactions also were noted in writing on a report sheet prepared for that purpose.

Most of the patients selected for treatment were those with the heaviest hookworm infections, with the average Stoll counts in excess of 2,000 eggs per gram of feces. However, 14 of the patients had egg densities ranging from 1,000 to 1,900 per gram. A group of 39 with egg densities from 50 to 1,100 per gram was specially selected for a comparison of the cure rate between high and low intensities.

In samples taken over a number of years, the adult hookworms recovered were identified as *N. americanus*. As no other species had ever been found here, it is assumed that all of the hookworm infections were *N. americanus*.

OBSERVATIONS

Two hundred nine patient-drug trials were made. There were 168 trials with bephenium hydroxynaphthoate and 41 with the tetrachloroethylene. The results of the bephenium trials are shown in Table 1.

Bephenium hydroxynaphthoate was tried against a total of 163 hookworm infections. It is

TABLE 1

Effect of bephenium hydroxynaphthoate upon worm infections

Regimen no.	Regimen (base)				Patient trials	Hookworm		Trichuris		Ascaris	
	Grams	Times/d	Total days	Total grams		C/T	% egg reduction	C/T	% egg reduction	C/T	% egg reduction
1	2.5	1	1	2.5	12	0/12	67	0/12	32	—	—
2	5.0	1	1	5.0	24	3/21	71	0/22	7	4/4	100
3 (LD)	5.0	1	1	5.0	13	3/13	70	0/11	24	—	—
4	2.5	3	1	7.5	13	1/12	75	1/12	37	1/1	100
5	5.0	1	2	10.0	9	1/8	82	0/7	42	2/2	100
6	2.5	2	2	10.0	9	1/9	74	0/9	53	—	—
7	5.0	1	3	15.0	18	7/18	95	1/17	46	1/2	99.6
8 (LD)	5.0	1	3	15.0	17	9/17	76	2/13	8	2/2	100
9	5.0	1	4	20.0	14	8/14	97	4/14	42	—	—
10	5.0	1	5	25.0	17	11/17	98	4/16	65	2/2	100
11 (LD)	5.0	1	5	25.0	9	8/9	91	3/9	82	—	—
12	5.0	1 (wkly)	2 wks	10.0	7	0/7	76	0/7	45	—	—
13	5.0	1 (wkly)	3 wks	15.0	6	3/6	84	1/6	21	—	—

(LD) = Hookworm infections of low density; Times/d = Times per day; C/T = Cured/Treated; wkly = weekly; wks = weeks.

obvious that an increase in the total dose of the drug produced a proportional increase in the effect against the hookworm infection. While a single dose of 5 grams for 1 day produced an egg reduction of 71%, only 3 cures were effected in 21 cases. When 5 grams daily for 2 days were given (regimens 5 and 6, Table 1) there was a slightly greater egg reduction. In groups of patients with high density infections receiving dosages of 15 or more grams at the rate of 5 grams single dose per day, the egg reduction was from 95 to 98%, with 26 of 49 being cured (regimens 7, 9, and 10, Table 1).

A single weekly dose of 5 grams of bephenium administered for 2 and 3 weeks (Table 1) did not appear to be superior to the same number of consecutive daily doses. A comparison of administration of the drug in divided doses (2.5 grams b.i.d. × 2 days) with single daily doses (5.0 grams daily × 2 days) indicated no great difference in effect in a limited number of trials (9 and 8 cases, respectively, regimens 5 and 6, Table 1).

Because most of the patients treated with bephenium had relatively high hookworm egg counts, patients with low egg counts were treated with representative dosages to determine if higher cure rates might be obtained in the latter group. Table 2 presents the comparison between the high and low egg density groups treated with total doses of 5, 15, and 25 grams of bephenium. In the low intensity hookworm group, the reduction of eggs was slightly lower and the cure rate slightly higher than in the heavier infected group, but the differences do not appear to be important.

In addition to the hookworm, most of the patients also had *Trichuris trichiura* infections (Table 1). These infections had a wide range of intensity. Most of the infections could be considered heavy as 83% had egg densities in excess of 2,000 per gram; 46% exceeded 10,000 eggs per gram. As can be seen in Table 1 the different dosages exerted various effects on this worm but in doses totaling 15 or more grams of base the reduction was often substantial, with 14 of 69 infections being eliminated.

Twelve out of 13 *Ascaris lumbricoides* infections treated with bephenium were eliminated (Table 1) and the remaining one reduced by 99.6%. Of the two known cases of *Enterobius vermicularis* in this group, neither was cured. A single case of *Hymenolepis nana* treated with 7.5 grams of bephenium in a single day was not cured.

Of 169 patients receiving bephenium, side effects were noted in 12 as follows: nausea 1, vomiting 9, diarrhea 2. In one case the vomiting was considered severe enough to discontinue the drug (this patient was dropped from the study); in others the vomiting usually occurred once or

TABLE 2

The comparative effect of bephenium hydroxynaphthoate treatment of hookworm cases with high and low egg counts

Regimen†			Low density cases				High density cases			
Daily dose	Days	Total dose	Pre-Rx egg counts* Range	Mean	% egg reduction	C/T	Pre-Rx egg counts* Range	Mean	% egg reduction	C/T
5.0	1	5.0	1–10	5	70	3/13	15–200	64	71	3/21
5.0	3	15.0	0.5–11	5	76	9/17	13–366	65	95	7/18
5.0	5	25.0	1–6	4	91	8/9	12–181	54	98	11/17

* = Egg counts in 100's per gram of feces.
† = In grams of base.
C/T = Cured/Treated.

TABLE 3

Response of helminths to tetrachloroethylene

Regimen			Hookworm				Trichuris			
Dose	Days	Total	Pre-Rx egg count* Range	Mean	% egg reduction	C/T	Pre-Rx egg count* Range	Mean	% egg reduction	C/T
3 ml (with purges)	1	3 ml	12–209	114	49	0/7	23–137	65	†	0/7
3 ml (without purges)	1	3 ml	21–126	50	58	0/7	1–57	30	10	0/4
4 ml	1	4 ml	9–256	54	51	1/22	3–308	79	16	0/26

* = Egg counts in 100's per gram of feces.
† = Increase in egg count.
C/T = Cured/Treated.

twice during a 3 to 5 day course of the drug. In most of these cases another dose was immediately administered successfully and the regimen completed. There was no apparent pattern to the vomiting and no apparent relationship to total dose or frequency of administration. In two groups receiving the higher dosage several patients vomited after being allowed to exercise in a very hot sun soon after taking the drug and this may account for the rather large percentage who vomited.

Tetrachloroethylene, one of the standard drugs for the treatment of hookworm, was given to 41 patients in three dose regimens for comparison. The response of the helminths to this treatment is shown in Table 3. The 3-ml single dose treatments were slightly better without than with a post-treatment purge, the egg counts being reduced by 58% and 49%, respectively. The 4-ml single dose treatment without a post-treatment purge reduced the hookworm burden by 51% and cured only 1 of 22. The *T. trichiura* egg density was reduced by 16%, with no infections eliminated. Three of five *E. vermicularis* infections were eliminated. There was no nausea, vomiting, or other side effects observed following use of this drug.

DISCUSSION

Bephenium hydroxynaphthoate appears to be an efficient drug against hookworm. The granular form (Alcopara®) is easily administered suspended in milk, fruit juices, or other liquids. When used against relatively heavy infections in single daily 5-gram doses for 3, 4, or 5 consecutive days, 95% or more of the hookworms were removed and about one-half of the patients were cured.

The reasons for the differences between the rates of reduction in egg densities and the lower cure rates are not known. It may be that some of the worms are in places not readily accessible to the drug.

The drug showed excellent effect against *A. lumbricoides*, curing 12 out of 13 infections and virtually curing the remaining one. The higher dosages reduced the *T. trichiura* infections substantially, and 15 grams or more eliminated some of the infections.

Under similar conditions practically all of the bephenium regimens, even those as low as 2.5 grams single dose, had superior anthelmintic action compared with the regimens of tetrachloroethylene tried. In addition to removing more worms, bephenium appears superior to tetrachloroethylene in that no fasting, purges, or other changes in the normal habits are required. Because of its action against *A. lumbricoides* and other worms, it is useful in cases with multiple infections.

The results obtained with bephenium hydroxynaphthoate are in general agreement, within comparable dose ranges, with those of Goodwin *et al.* who treated *N. americanus* infections in Ceylon.[2] Their dosages of from 2 to 3 grams base reduced egg densities from 64 to 78% whereas our reduction was 67% at the 2.5-gram dose. Their 6-gram total for one day gave 79% reduction whereas we obtained 70 to 75% in the 5.0 to 7.5-gram range.

The report of Nagaty and Rifaat[3] shows a considerably greater cure rate than we have seen. Using a single dose of 2.5 grams base they were able to cure about 95% of *Ancylostoma duodenale* infections in 239 individuals. Using this dosage in 12 infections with *N. americanus* we produced no cures and only a 67% reduction in worm burden. It is possible that a difference in parasite species might account for the difference in drug effect.

In view of the results, it appears that in mass treatment a satisfactory reduction of hookworm infections might be obtained by single doses of as little as 5 grams. For patients who can be treated for several days, 5 grams daily for 3, 4, or 5 days could be given. The 3-day treatment possibly is the one of choice, combining an intermediate length of therapy with a relatively high reduction of infection.

SUMMARY

The hydroxynaphthoate salt of bephenium was tried 168 times against worm infections in mental patients. Tetrachloroethylene was tried 41 times for comparison.

Bephenium hydroxynaphthoate, in granular form (Alcopara®), exerted good effects against hookworm (*Necator americanus*). It was more effective than tetrachloroethylene. Dosages of 5.0 grams base single dose daily for 3 or more consecutive days reduced heavy hookworm infections by 95% or more with about 55% of the infections cured. Five-gram doses for 1 or 2 days greatly reduced the hookworm burden but the cure rate was low. The optimum regimen appears to be 5 grams single dose for 3 days or, for mass therapy, a single 5-gram dose.

The drug was highly effective against *Ascaris lumbricoides*, giving an over-all egg reduction of over 99%; 12 of 13 infections were eliminated.

Bephenium also was moderately effective against *Trichuris trichiura* infections. Dosages totaling 15 or more grams resulted in a substantial reduction in worm burden and produced some cures.

Of two known *Enterobius vermicularis* infections treated, neither was cured. A single *Hymenolepis nana* infection was not eliminated.

Side effects, principally vomiting, occurred in about 7 per cent of the patients. However, in only one patient was it necessary to discontinue treatment.

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EFFICACY OF BEPHENIUM AND TETRACHLOROETHYLENE IN MASS TREATMENT OF HOOKWORM INFECTION*

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Since tetrachloroethylene was recommended by Hall and Shillinger[1] for the treatment of hookworm infection, it has come to be accepted widely as the drug of choice, but there has been considerable disagreement as to how effective it really is. Many of our patients received this drug with benefit but with few cures, and considerable skepticism concerning the excellent results reported by Carr and his co-workers[2] seemed justified. In Georgia, however, the results obtained with tetrachloroethylene without purgation appeared encouraging in comparison to those obtained with the other available anthelmintics.

When bephenium hydroxynaphthoate was reported by Goodwin *et al.*[3] to be safe and effective in the treatment of hookworm infection it appeared to many that a drug to supersede tetrachloroethylene had at last appeared. Early in 1959 the opportunity presented itself to compare the efficacy of tetrachloroethylene and bephenium in a mass treatment program against hookworm in Lowndes County, in south central Georgia.

METHODS

An attempt was made to examine a stool specimen from each child at the seven elementary schools in the county. These schools are attended by children 6 to 17 years old comprising all of the white rural children of that age in the county. Approximately 4,500 urban children were omitted from the study. Of the 2,963 rural children, 54.17% (1,807) co-operated by submitting stools for examination. Stools were examined by the brine flotation method and if positive, eggs were counted by the direct smear method.[4] Stools were examined on the same day passed, and therefore no preservative was necessary.

Children found to be infected were offered treatment. Thirty-nine children declined treatment but consented to submit additional stools for examination.

The following treatment regimens were used: (1) 5 grams Alcopara® granules† (= 2.5 grams bephenium base) three times daily 2 hours after meals. (2) 5 grams Alcopara granules (= 2.5 grams bephenium base) twice daily 2 hours after meals. (3) Tetrachloroethylene 0.06 ml per pound of body weight with a maximum dose of 5.0 ml. No purgative was given. The Alcopara granules were given as a suspension in water.

Drugs and instructions for taking them were distributed to the children at the schools and the doses were administered by the parents at home. Parents were instructed to telephone the health department to report untoward symptoms or to ask questions, and a record was kept of all calls. During the week following treatment a questionnaire was submitted to both treated and untreated children to elicit information concerning side effects of the drugs used.

Beginning 2 weeks after treatment stools were again submitted to determine the effect of treatment. From one to five post-treatment specimens were submitted over a period of 3 weeks. Methods of handling the post-treatment specimens were exactly the same as before, except that 80 post-treatment specimens mailed to New Orleans were preserved with phenol. Results did not indicate any significant difference between these and the unpreserved specimens.

RESULTS

Degree of co-operation. A single pre-treatment stool specimen was examined from each of 214 children. During the period after treatment 21 children each submitted one specimen, 43 two specimens, 125 three specimens, 24 four specimens and 1 five specimens. These include 39 untreated children observed for comparison.

Side effects. Side effects were obtained with both tetrachloroethylene and bephenium but were more common with bephenium (Table 1). Five of the eleven tetrachloroethylene patients experiencing symptoms reported nausea only.

* This study was supported by a grant provided by Burroughs-Wellcome & Co. (U.S.A.) Inc.

† A flavored powder containing bephenium hydroxynaphthoate, provided through courtesy of Burroughs-Wellcome & Co. (U.S.A.) Inc.

TABLE 1

Symptoms reported by 51 of 175 children treated with tetrachloroethylene or bephenium for hookworm infection

Symptoms	Bephenium	Tetrachloroethylene	Total
Nausea only	4	5	9
Vomiting only	16	1	17
Diarrhea only	0	0	0
Abdominal pain only	2	0	2
Nausea, vomiting	13	0	13
Nausea, abdominal pain	0	1	1
Nausea, vomiting, diarrhea	1	1	2
Nausea, vomiting, abdominal pain	3	0	3
Staggering	0	1	1
Weakness	0	1	1
Could not swallow drug	1	1	2
Total with side effects	40 (42%)	11 (14%)	51
Number treated	95	80	

Vomiting and nausea were the most common symptoms caused by treatment with bephenium. The fact that vomiting was more common than nausea is related to the fact that several children regurgitated the dose immediately after swallowing as a consequence of its unpleasant taste; these children did not complain of nausea. Nausea was also caused by tetrachloroethylene, but staggering seen in one child treated with tetrachloroethylene was not caused by bephenium.

Since treatment was given on Saturday and Sunday, attendance records at the schools were checked on Monday to determine whether treatment with one or the other drug had resulted in absence from school. Analysis of these results indicated that there was a higher rate of absences among children treated with either drug than among the rest of the children, but neither drug was associated with more absenteeism than was the other (Table 2).

Total number treated is greater in Table 2 than in Table 1 since the former includes individuals who dropped out of the study after treatment.

No symptoms were reported by the 39 untreated children studied in detail, although among the schools concerned in the study there was a 6.9% absentee rate among untreated children.

TABLE 2

Absences of children from school on the Monday following treatment of hookworm infection with bephenium or tetrachloroethylene

Treatment	Absent	Not absent	Total
Tetrachloroethylene	15 (12.7%)	103	118
Bephenium	20 (13.3%)	130	150
Not treated	154 (6.9%)	2079	2233
Total	189 (7.6%)	2312	2501

TABLE 3

Results of treating 175 children with bephenium or tetrachloroethylene and observations on 39 untreated children

Treatment class*	Total cases	Number cleared	Per cent cleared
Untreated	39	10	25.6
Tetrachloroethylene	80	65	81.3
Bephenium × 3	24	7	29.2
Bephenium × 2	40	12	30.0
Bephenium × 1	31	5	16.1
Total	214	99	46.2

$\chi^2 = 64.3$, $P < .001$.

Tetrachloroethylene vs untreated, $\chi^2 = 34.89$, $P < .0005$; bephenium × 3 + bephenium × 2 vs untreated, $\chi^2 = 0.2$, $P > .30$.

* Tetrachloroethylene = 0.06 ml per pound up to 5.0 ml; bephenium × 3 = 2.5 grams 3 times in 1 day; bephenium × 2 = 2.5 grams twice in 1 day; bephenium × 1 = 2.5 grams taken once.

Because of the inability of some children to tolerate bephenium many of those scheduled to take three doses took only one or two, and some scheduled for two doses took one. These changes resulted in there being the following groups for final analysis: (1) tetrachloroethylene 0.06 ml per pound up to 5.0 ml; (2) bephenium 2.5 grams three times in 1 day; (3) bephenium 2.5 grams twice in 1 day; (4) bephenium 2.5 grams taken only once; (5) untreated.

Worm burden. The worm burden was generally low. Of the 214 children studied before treatment 98 (45.8%) passed 1 egg or less per 2 mg of feces; 158 (73.8%) 5 eggs or less; and 174 (81.3%) 10 eggs or less.

Efficacy. Table 3 shows the disappearance of

TABLE 4

Reduction in hookworm egg count in 214 children who were untreated, treated with tetrachloroethylene or with one, two, or three doses of bephenium

	Mean egg count (eggs/2 mg)*		Per cent reduction	No. children in group
	Before	After		
Untreated	7.00	3.48	50.2	39
Tetrachloroethylene	6.45	0.06	99.1	80
Bephenium × 3	1.67	0.18	89.2	24
Bephenium × 2	13.45	2.12	84.2	40
Bephenium × 1	5.26	1.90	63.8	31

* Egg counts of zero were given a value of zero in calculating the mean when eggs were found by brine flotation.

eggs from stools of treated children as compared with the untreated ones. Only with tetrachloroethylene treatment was there clearance of infection (81.3%) significantly greater than that observed in untreated children (25.6%). Two and three doses of bephenium produced similar clearance rates (29.2% and 30.0%, respectively), not significantly different from the untreated group.

In all groups, treated or not, there was an apparent decrease in egg count during the post-treatment period (Table 4). This reduction was greatest in those treated with tetrachloroethylene. The degree of reduction in children treated with bephenium was greatest in those given three doses, and least in those given a single dose. The differences in reduction between untreated children and those receiving tetrachloroethylene or bephenium were significant.

DISCUSSION

Side effects, especially nausea and vomiting, were obtained frequently with bephenium. This is in contrast with published and unpublished reports of others. The possible explanations for greater intolerance of the drug here are: (1) Parents were told in advance to call the Health Department if trouble was encountered. A form report on all treated children was requested. (2) This group was composed entirely of American Anglo-Saxon juveniles as compared with other studies involving adults and other racial and cultural groups. (3) The persons in the present study live in a district where treatment with tetrachloroethylene is now being carried out on the third generation. They are accustomed to receiving tetrachloroethylene capsules, and the use of bephenium powder may have aroused suspicion in the parents.

Reports of side effects from tetrachloroethylene were elicited for the first time. Prior to this study the Georgia Department of Public Health had received no reports of side effects from the routine use of tetrachloroethylene without purgation.

There was an apparent reduction in egg count regardless of treatment. Chandler[5-7] reported the spontaneous diminution of hookworm burdens in human infections over a period of a few months in which there was no opportunity for reinfection. Similar observations over a long period have been made by Kendrick.[8] Caldwell and Caldwell[9] in a 6-month study of a small group of children in rural Alabama observed a rise in mean egg count between October and January, and a decline thereafter until April. Nevertheless they concluded that the worm burden was stable. Cort *et al.*[10] on the other hand found no fluctuation in egg counts made during the rainy and dry seasons in Panama and attributed the differences between their observations and Chandler's to the lighter infections in the latter's study group.

It is more probable that the reduction in egg count was in part apparent and not real. In the population of children studied hookworm infection was found in some and not in others, but in almost all cases the infections were light. It is proper to assume that the examinations sometimes failed to detect eggs in the stools of lightly infected children, and at other times did not. Those whose infections were not discovered during the preliminary screening were eliminated from the study. Those whose infections were not revealed during the follow up period appeared to be "cured" or to have spontaneously lost the infection.

Had an untreated group not been studied for comparison with the treated groups, the two drugs each would have appeared to be more effective than it is safe to assume on the basis of the data obtained. Failure to provide a similar untreated group in other clinical tests may be responsible for enthusiastic reports concerning drugs which later are disappointing.

Efficacy of the drugs in question was calculated on two bases—clearing and reduction of infection. By statistical analysis tetrachloro-

ethylene was shown to be effective in both regards. Bephenium administration was associated with a significant reduction in egg count but not in significant clearing of infection. Nevertheless results with bephenium in this study are remarkably similar to those of Goodwin *et al.*[3] in Ceylon, who concluded that the drug is satisfactorily effective in both respects. The different conclusion in the present study is a result of the rate of apparent spontaneous loss of infection in untreated individuals.

The merit of the statistical analysis is decreased by the following factors: (1) The untreated group is not a true control since the individuals were selected not at random but by their refusal of treatment. Refusals were for religious reasons or because of parental inertia. Spontaneous loss of worms in these individuals seemed unlikely to be inordinate and they were therefore included for comparative purposes when it was found impractical to select an untreated group at random. (2) Variability of worm burden from one treatment group to another casts some doubt on validity of comparisons.

The latter factor was considered in advance of the study, and it was planned that each group would contain children with comparable egg counts. Unfortunately most of the children who did not tolerate three doses of bephenium had higher egg counts and their transfer into the group receiving only two doses raised the mean egg count of the latter group and lowered that of the former.

As has been observed many times with regard to other diseases efficacy of methods of treatment of hookworm infection cannot be evaluated without comparison of results in treated and untreated individuals.

SUMMARY

Of 214 children with hookworm infection in Lowndes County, Georgia, 80 were treated with tetrachloroethylene, 95 with one, two, or three doses of bephenium, and 39 were not treated. Following treatment, percentage reductions in mean egg counts were approximately 99, 89, 84, 64 and 50, respectively; and percentages of children completely cleared were 81, 29, 30, 16 and 26, respectively. It was concluded that bephenium possessed some anthelmintic activity but that tetrachloroethylene was superior. Side effects were more commonly produced by the bephenium preparation than by the tetrachloroethylene.

ACKNOWLEDGMENT

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THE TREATMENT OF HOOKWORM, *ASCARIS* AND *TRICHURIS* INFECTIONS WITH BEPHENIUM HYDROXYNAPHTHOATE

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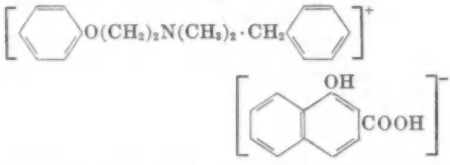
Bephenium hydroxynaphthoate was recently found to be effective against *Necator americanus* and *Ascaris lumbricoides* infections by Goodwin *et al.*[1] and Gunders.[2] They observed no toxic side effects, even when large doses of this compound were administered. In view of the frequency of multiple helminth infections in many areas, broad-spectrum anthelmintics that are safe, effective and cheap are greatly needed. We therefore evaluated the efficacy of bephenium hydroxynaphthoate on a group of patients harboring the most common helminth trinity, hookworm (*Ancylostoma duodenale* and *Necator americanus*), *Ascaris lumbricoides* and *Trichuris trichiura*, in Taiwan.

A number of other bephenium compounds are under investigation in laboratory animals and man but the hydroxynaphthoate appears, at present, to give the most promise.[1-3]

MATERIALS AND METHODS

Bephenium hydroxynaphthoate** is a quaternary ammonium compound. It is marketed in England under the name of "Alcopar." The structural formula is as follows:



The majority of the patients selected for the clinical trials were children 10 to 15 years of age. They were admitted to the hospital for therapy and the drug was administered to them by the authors. Usually the drug was dissolved in water; however, some patients preferred to place the drug in their mouth and wash it down with water. The drug is somewhat bitter tasting. *All dosages of bephenium hydroxynaphthoate are in terms of base.* The treatment regimes varied from 1 to 7 days, and from 2 to 5 grams of bephenium hydroxynaphthoate (base) was administered daily.

The degree of parasitic infection was measured by the Stoll dilution egg-count technique (uncorrected to formed stool basis). In the post-treatment stool examination, which was done 1 month after treatment, if no eggs were found in the Stoll count, concentration employing the formalin-ether technique was done to confirm cure of the infection. For hookworm, larval cultivation was also made by the method developed by Sasa *et al.*[4] in order to detect light infections. Filariform larvae cultivated for 8 to 10 days under 22 to 25°C were examined to differentiate *A. duodenale* from *N. americanus*. The post-treatment stools of 11 of 48 patients were also carefully washed and the worms collected.

RESULTS

Forty-four of the 48 patients treated harbored *Ascaris*, 48 harbored *Trichuris*, and 48 harbored hookworm. This enabled us to compare the efficacy of the drug on the three parasites in the same host. Of the 48 patients harboring *Ancylostoma* only 8 of this group also harbored *Necator* and hence our data refer largely to the former hookworm.

Hookworm

Bephenium hydroxynaphthoate proved to be very effective against hookworm infections which were predominately *Ancylostoma* (Table 1). The egg-count reduction was over 99% in each group of patients treated from 1 to 7 days. The single-day treatment regime of two doses of 2.5 grams (base) each removed 99% of the worms and cured 74% of the 23 patients. Four patients in this group harbored both *Ancylostoma* and

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** Supplied by Burroughs Wellcome & Company, U.S.A.

TABLE 1

Efficacy of bephenium hydroxynaphthoate against hookworm, Ancylostoma duodenale *and* Necator americanus

Patients	Kg weight	Dosage (as base)			Total egg count/ml feces			Per cent patients cured
		Grams	Per diem	No. days	Before therapy	After therapy	Per cent reduction	
2	36–45	2.5	1	5	2,800	0	100	100
6	31–58	2.5	1	7	23,200	200	99.9	83
17	21–38	1–1.5	2	2	33,000	200	99.9	64
23	20–44	2.5	2	1	103,600	1,400	99.0	74

TABLE 2

Efficacy of bephenium hydroxynaphthoate against Ascaris lumbricoides

Patients	Kg weight	Dosage (as base)			Total egg count/ml feces			Per cent patients cured
		Grams	Per diem	No. days	Before therapy	After therapy	Per cent reduction	
2	36–45	2.5	1	5	205,100	11,800	95	50
5	31–58	2.5	1	7	196,000	4,800	97	80
17	21–38	1–1.5	2	2	662,700	152,900	77	18
20	20–34	2.5	2	1	973,400	89,000	91	65

Necator; and it is of interest that whereas all of the *Ancylostoma* were removed, three of the patients still harbored *Necator* after therapy. Nineteen of this group of 23 harbored only *Ancylostoma*. In this group the egg-count reduction was 99.8% and the cure rate 89%.

All stools of the 8 patients, treated for 5 or 7 days, were carefully searched for adult hookworms the first 4 days of therapy. A total of 302 hookworms was recovered, 220 on the 1st day, 77 on the 2nd day, 5 on the 3rd day, and none on the 4th day.

Ascaris

Forty-four patients harboring *Ascaris* were treated with bephenium hydroxynaphthoate for 1 to 7 days (Table 2). A single dose of 2.5 grams for 5 or 7 days resulted in egg-count reduction of 95 to 97%. When the dose was reduced to 1.0 to 1.5 grams twice daily for 2 days, the egg-count reduction dropped appreciably and the cure rate was markedly decreased. By contrast, when approximately the same amount of drug was given on 1 day instead of being divided over 2 days, a highly satisfactory egg-count reduction of 91% and a cure rate of 65% were achieved.

The worms passed in the stool, as a result of the 5- to 7-day course of therapy, were collected for the first 4 days; 24, 103, 8, and 18 were passed.

Trichuris

Bephenium hydroxynaphthoate in the dosages employed was moderately active against *Trichuris* (Table 3). Egg-count reductions of 25% to 59% were achieved, but the cure rates were low in the two groups of patients that were large enough to give significant results. In view of the 59% reduction in egg count in the group of patients given a total of 5.0 grams of the base on 1 day, it would appear that further studies are indicated to establish the most effective therapeutic regime. A total of 26 worms was collected from the group of patients treated for 5 to 7 days, 19 on the 2nd day of therapy, and 7 on the 3rd day.

Side Effects

Bephenium hydroxynaphthoate has a bitter taste, yet it is acceptable to both children and adults. Daily doses of 2.5 grams (base) for 5 to 7 days caused some dizziness, nausea, vomiting, abdominal discomfort, diarrhea and headache in children. These side effects were of sufficient severity and frequency to limit the use of this

TABLE 3

Efficacy of bephenium hydroxynaphthoate against Trichuris trichiura

Patients	Kg weight	Dosage (as base)			Total egg count/ml feces			Per cent patients cured
		Grams	Per diem	No. days	Before therapy	After therapy	Per cent reduction	
2	36–45	2.5	1	5	4,000	1,000	25	0
6	31–58	2.5	1	7	14,600	8,000	45	33
17	21–38	1–1.5	2	2	33,900	24,700	27	0
23	20–44	2.5	2	1	51,800	21,400	59	4

therapeutic regime in mass therapy. When the dosage was reduced to 1.0 to 1.5 grams of the compound twice daily for 2 days or 2.5 grams twice daily for 1 day, the side effects were greatly reduced, and consisted of only occasional vomiting and headache, diarrhea and abdominal discomfort. All side effects quickly subsided when the drug was discontinued.

DISCUSSION

Bephenium hydroxynaphthoate is highly effective against *Ancylostoma* and *Ascaris* in man. It is moderately effective against *Trichuris*. Although daily doses for 7 days are most effective, the side reactions due to the drug are likewise most frequent. Bephenium hydroxynaphthoate, however, is highly effective when given for only 1 day in total amounts of 5.0 grams (base). Using this regime 89% of the *Ancylostoma*-infected patients were cured and their total egg count was reduced 99.8%. Nagaty and Rifaat[5] recently reported a 95.4% cure rate in 239 *Ancylostoma*-infected patients given a single dose of 2.5 grams (base) of this drug. Ahmad and Ghulam[6] achieved a cure rate of 83% in the 74 patients who harbored both *Ancylostoma* and *Necator*, the former predominating. Sixty-five per cent of the *Ascaris*-infected patients were cured and their total egg count reduced 91%. There is no single, safe anthelmintic with equal efficacy against both *Ancylostoma* and *Ascaris*. The drug is only moderately effective against *Trichuris*.

Our studies indicate that bephenium is more active against *Ancylostoma* then *Necator* which is unusual, for the former hookworm has proven to be more resistant to removal by anthelmintics than has the latter. Hsieh *et al.*[7] note that tetrachloroethylene and 1-bromo-naphtho-(2) are not as effective against *Ancylostoma* as they are against *Necator*. Other studies likewise indicate that tetrachloroethylene is not as effective against *Ancylostoma* as it is against *Necator*. This is a possible advantage of bephenium.

Gunders[2] treated a group of *Necator*-infected patients, the majority of whom were adults, with 5.0 grams of the drug (base) on 1 day. He achieved an egg-count reduction of 67% and only 16% of the patients were cured of their infection. Comparing these results with ours suggests that bephenium is more effective against *Ancylostoma* than *Necator*. However, as the majority of our *Ancylostoma* patients were children and Gunders' *Necator*-infected patients were adults, and as both groups received a total of 5.0 grams of the drug (base), our *Ancylostoma*-infected group received a larger dose per kilogram of body weight. However, the apparent greater efficacy of the drug against *Ancylostoma* may not have been entirely due to the actual difference in per kilogram dosage, for Goodwin *et al.*,[1] who gave 6.0 grams (base) on 1 day to a group of adults, reduced their *Necator* egg count only 79%. The relative value of bephenium hydroxynaphthoate against *Necator* alone must be viewed in the light of the report of Carr *et al.*,[3] who removed 92% of the *Necator* from a large group of adults with a single, safe, cheap dose of tetrachloroethylene.

The excellent results reported[6] of the effect of bephenium against *Trichostrongylus*, with 82.6% of a group of 23 patients cured following a single dose of 2.5 grams (base), suggest that at last we have an active agent against this persistent parasite.

Extensive toxicological studies (personal communication, Burroughs & Wellcome Company, U. S. A.) on mice, rats, monkeys, and dogs have failed to demonstrate hematological, renal, or hepatic damage from relatively large daily

doses of bephenium hydroxynaphthoate, given over periods as long as 4 months. Careful pathologic studies confirmed the lack of damage to the animals' tissues by the drug. The LD_{50} dose for mice is 8 grams (base) per kilogram of body weight. Rogers[9] has demonstrated that very small amounts of bephenium hydroxynaphthoate and its metabolites are excreted in the urine.

The bitter taste of bephenium hydroxynaphthoate is difficult to mask and may preclude an easily administered liquid preparation. The side effects produced by the drug, when administered for 5 to 7 days, are markedly reduced by the single-day therapeutic regime. For mass therapy, the 1-day regime has obvious advantages.

SUMMARY

Bephenium hydroxynaphthoate administered to children 10 to 15 years of age in total amounts of 4.0 to 17.5 grams (base) over a period of 1 to 7 days proved to be highly effective against *Ancylostoma duodenale* and *Ascaris lumbricoides*. It was less effective against *Necator americanus* and *Trichuris trichiura*. The side effects from the 5- and 7-day courses of therapy were of sufficient severity and frequency to limit the use of these therapeutic regimes in mass therapy. However, when a total of 5.0 grams of the base was given in two doses on 1 day, the side reactions were not significant or extensive and the *Ancylostoma* and *Ascaris* cure rates were excellent. This latter regime appears to be the most desirable one at present, although it is possible that further study may show that 1-day therapy with doses smaller than 5.0 grams is effective. Pharmacologic studies indicate that this drug is safe in therapeutic dosage.

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THE TREATMENT OF ENTEROBIASIS WITH NEW FORMULATIONS OF DITHIAZANINE IODIDE*

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There has been a justifiable interest in new formulations of dithiazanine iodide§ because of a significant incidence of side effects associated with the use of the presently available commercial tablets. Side effects observed with therapeutic doses have consisted of anorexia, nausea, vomiting, abdominal cramps, diarrhea, dizziness, and lethargy.[1-10] F. G. Henderson and his associates in the Lilly Research Laboratories have demonstrated that when dithiazanine iodide comes in contact with the gastro-intestinal mucous membrane, hypermotility ensues in the segment where the contact occurs. This experiment in animals suggests that the side effects produced by dithiazanine iodide are due to chemical irritation. No morphological changes in the mucous membrane have been produced, however, in therapeutic dosage levels in either animal or man. Possible solutions to this problem naturally suggest themselves, such as special protective coatings, methods of slow-release of the drug near the site of the intestinal parasites, and the use of salts of dithiazanine with different solubility characteristics than those of the iodide.

The present study consists of a clinical trial of four modifications of the commercial tablet of dithiazanine iodide. The therapeutic effects and incidence of side effects are compared with those observed with the commercial tablets in the treatment of a group of individuals infected with *Enterobius vermicularis*.

MATERIALS AND METHODS

Patient material consisted of teen-age boys and girls institutionalized because of delinquency. Subjects were housed in cottages accommodating 30 to 50 individuals. All the inhabitants of each cottage were examined by the cellulose tape method at the same time on 3 successive mornings. All those found positive were treated simultaneously. Treatment was carried out by a registered nurse. Each time the dose was administered, she questioned the patients regarding side effects noted since the previous dose. All medications were administered 3 times daily, approximately 1 hour after meals, for a 5-day period. Post-treatment cellulose tape preparations were begun on the 3rd day after the termination of treatment and continued for 5 successive mornings. An "apparent cure" was reported if all five cellulose tape preparations were negative for ova of *E. vermicularis*. A treatment failure was reported if any one of the slides contained one or more ova.

The commercial tablets consisted of a core of dithiazanine iodide and starch, moderately compressed and coated with a film of cellulose phthalate.

"Dithiazanine I" consisted of a modification of the commercial tablet. Methylcellulose was added to the core of the tablet and the cellulose phthalate film was thickened by approximately 150%.

"Dithiazanine II" was the same as "Dithiazanine I" except that the thickness of the cellulose phthalate coating was identical to that of the commercial tablets.

"Dithiazanine III" was identical to "Dithiazanine II" except that the polyvinyl pyrrolidone was substituted for the methylcellulose in the tablet core.

"Dithiazanine IV" was identical to "Dithiazanine II" except that a special procedure was used to modify the size of the dithiazanine iodide and methylcellulose granules.

Both methylcellulose and polyvinyl pyrrolidone tend to act as a cement substance, delaying tablet disintegration. A tablet dissolving slowly as it passed through the gut would distribute its contents over a longer segment than would a rapidly dissolving one, resulting in a relatively greater dilution of the medicament.

The commercial tablets were compared with the modified tablets by determining the disintegration time in artificial gastric and intestinal fluids with a Stowl-Gershberg (Vanderkamp)

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‡ The Lilly Research Laboratories, Indianapolis.

§ Delvex® is the trademark of Eli Lilly and Company for their product, dithiazanine iodide.

TABLE 1

Results with various formulations of Delvex® tablets in a 5-day treatment of enterobiasis

Formulation	Daily dose	Number treated	Apparent cures	Incidence of side effects	Patients vomiting once only	Patients vomiting twice or more
Commercial tablets	300 mg	16	16	13	7	3
Commercial tablets	150 mg	27	23	15	3	2
"Dithiazanine I" thicker coating, methylcellulose core	300 mg	21	21	5	0	0
"Dithiazanine II" methylcellulose core	300 mg	23	22	4	1	0
"Dithiazanine III" PVP core	300 mg	21	20	9	0	1
"Dithiazanine IV" methylcellulose core with special granulation	300 mg	16	16	6	2	1

U.S.P. apparatus. All types of tablets administered in this study remained intact in simulated gastric juice after several hours. In simulated intestinal fluid at a pH of 7.5, the commercial tablets disintegrated in approximately 15 minutes. The modified tablets disintegrated as follows: "Dithiazanine I," 60 to 120 minutes; "Dithiazanine II," 60 to 90 minutes; "Dithiazanine III," 45 minutes; and "Dithiazanine IV," 45 minutes.

It may be that the disintegration time under these artificial conditions does not correspond to the actual disintegration time in the human intestine. However, the values are believed to be proportional and therefore useful as a means of comparison.

The modified tablets were identical to those used by others in the treatment of helminthiasis. The designations are identical to those used by Febles *et al.*[11] Tablets II, III, and IV correspond to Tablets A, B, and C used by Evans *et al.*[12]

RESULTS

Results obtained with the various formulations of dithiazanine iodide tablets are summarized in Table 1. When the commercial tablets were administered to 16 individuals at a dosage level of 100 mg t.i.d., all were apparently cured. Thirteen of the individuals had some side effects, 7 vomited at least once, and 3 vomited twice or more. When the dose was reduced to 50 mg t.i.d., all but four were apparently cured; 15 of the 27 subjects complained of side effects, 3 vomited once, and only 2 vomited twice or more. The differences in the incidence of all side effects, and of vomiting, are significant ($P = 0.001$). The four treatment failures with the reduced dosage are probably significant.

When the four tablet modifications were administered to groups of similar size at dosage levels of 100 mg t.i.d., all were apparently cured except one patient receiving "Dithiazanine II" and one receiving "Dithiazanine III." Abdominal cramping was the most common complaint among patients receiving "Dithiazanine IV" but was less common with the other tablet modifications. No patients receiving "Dithiazanine I" vomited; vomiting was observed in one patient receiving "Dithiazanine II," one "Dithiazanine III," and three "Dithiazanine IV." The total incidence of side effects and of vomiting among the patients receiving the special tablets is significantly less than that obtained with the commercial tablets in full dosage ($P = 0.001$) and half dosage ($P = 0.05$); the total incidence of side effects observed with "Dithiazanine II" is significantly less than that observed with the other special tablets. Otherwise, there are no significant differences in the incidence of side effects among the special tablets.

DISCUSSION

It is tempting to correlate the observed reduction in side effects with the prolonged disintegration time of these tablets in simulated intestinal fluid. Alvarez[13] has summarized the evidence that there is an intestinal gradient, the upper portion of the intestine being more active physiologically (hence possibly more reactive to chemical irritation) than the distal portion. The exact point in the intestine where the various types of tablets disintegrate is, however, as yet

undetermined. The reduced incidence of side effects with lower dosage of commercial tablets obtained in this study suggests that the incidence of side effects is related to the concentration of the drug in the intestinal contents. Hence, the slowing of the rate of dissolution of the tablets and the resulting greater dilution of the drug in the intestinal contents may be more important than the breaking up of the tablet at lower levels of the intestine.

SUMMARY

Various new formulations of tablets of dithiazanine iodide were administered to 81 teen-age individuals infected with *Enterobius vermicularis*. As compared with commercial tablets, administered to 43 patients, the new formulations gave evidence of improved patient tolerance without loss of therapeutic efficacy.

Commercial tablets at a dosage level of 300 mg daily for 5 days apparently cured all 16 subjects treated. Thirteen subjects complained of side effects, among whom 7 vomited once and 3 vomited twice or more. In a similar group receiving half the dose, the incidence of side effects was significantly reduced; but there were 4 treatment failures.

The incidence of side effects in the group receiving the four tablet-modifications was significantly reduced as compared with that in the group receiving the commercial tablets in full dosage or half dosage. There were two treatment failures. Best tolerance was noted in 23 patients receiving the tablets containing a methylcellulose core. Four had some side effects, among whom only one vomited once and none vomited twice.

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THE USE OF DITHIAZANINE IODIDE IN THE TREATMENT OF MULTIPLE HELMINTHIASIS IN SARAWAK, BORNEO

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Helminthiasis constitutes a major health problem in this part of the world. Routine stool examinations on 858 hospitalized patients during a period of 6 months revealed 59% of them to be infected with one or more of the following: *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, *Strongyloides stercoralis*, *Hymenolepis nana*, and *Enterobius vermicularis*. A new drug, dithiazanine iodide, has been introduced to the medical world as an effective broad-spectrum anthelmintic. Frye *et al.*[1] first showed its usefulness as a trichuricide. Swartzwelder *et al.*[2] demonstrated its broad-spectrum effect on trichuriasis, strongyloidiasis, ascariasis and enterobiasis. This drug was also found to have significant anthelmintic activity against human hookworm (*Necator americanus*) infection. Since then various workers have confirmed that this drug is a good trichuricide.[3, 4, 5, 6, 7] During the past 6 months dithiazanine iodide has been used on 35 patients in an attempt to find out its effect on hookworm (*A. duodenale*) infection, and its efficacy as a wide-spectrum anthelmintic to be used in mass treatment in this part of the world.

MATERIALS AND METHODS

Thirty-five patients, 21 male and 14 female, who ranged in age from 11 to 65 years and of whom 6 were Chinese, 1 Malay and the rest from native tribes such as Iban, Kenyah, Kayan, and Peng, were treated with dithiazanine iodide.* Ten were treated as outpatients and 25 as inpatients. Twenty-three of the 35 patients had triple infections with *A. lumbricoides*, *T. trichiura* and *A. duodenale*; 5 had *A. lumbricoides* and *A. duodenale*; 2 had *A. lumbricoides* and *T. trichiura*; 4 had *A. duodenale* and *T. trichiura*; and 1 had only *T. trichiura*. Dithiazanine iodide in the form of 100 mg enteric-coated tablets was given at a dosage of 200 mg three times daily after meals, for 1, 2, 3, 4, or 5 days. No special preparation was made before treatment and no purge was given after treatment. Stool egg counts by the Stoll technique were done on all patients before and after treatment. Post-treatment stools were examined usually 1 or 2 days after completion of treatment. Longer follow-up stool examinations were not possible once the patients left the hospital. No attempt was made to recover the expelled worms. A careful check of side reactions was made on all patients daily.

* Dithiazanine iodide employed in this study was supplied by Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

RESULTS

Trichuriasis. In 2 patients treated with dithiazanine iodide for 1 day at the dosage of 200 mg three times daily, there was 85% reduction in egg counts. After 2 days of treatment at the same dosage 2 of 7 patients showed no eggs in their stools, and there was 74% reduction in egg counts. In 1 patient treated for 3 days there were no eggs found in the stool. Of the 20 patients treated for 5 days 16 (or 80%) showed no eggs in their stools. There was an over-all 45% reduction in egg counts (Table 1).

Ascariasis. In 2 patients treated with dithiazanine iodide at a dosage of 200 mg three times daily for 1 day, there was no significant change in the egg counts. In 7 patients treated for 2 days, one showed no Ascaris eggs in the stool and the remaining showed a 43% reduction in egg counts. One patient treated for 4 days was found to have no more Ascaris eggs in the stool. In the remaining 18 patients treated for 5 days there was a reduction of 73% in egg counts, with 7 patients whose stools showed absence of Ascaris eggs. The over-all reduction in egg counts was 55% (Table 1).

Hookworm infection. Twenty-six patients with hookworm infections were treated with dithiazanine iodide at a dosage of 200 mg three times daily. Of these, 1 was treated for 1 day, 6 for 2 days, 3 for 3 days, and the remaining 16 for 5 days. Of the 16 patients treated for 5 days there was a total increase in the egg counts of 70%. Four patients whose stools were negative for hookworm eggs after treatment had low pre-treatment counts ranging between 100 and 300

TABLE 1

Ascariasis, trichuriasis, and hookworm infection treated with dithiazanine

Pt.	Dept.	Age	Dosage 200 mg t.i.d.	Trichuris egg counts/g		Ascaris egg counts/g		Hookworm egg counts/g	
				Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	InP	45	×1	700	200	13,600	10,200	1,700	1,300
2	InP	65	×1	4,100	500	216,600	255,600	0	300
3	OP	11	×2	—	—	500	2,300	300	0
4	OP	21	×2	700	300	5,700	500	700	200
5	OP	18	×2	2,600	0	16,100	3,600	900	100
6	OP	23	×2	1,500	0	—	—	—	—
7	OP	50	×2	1,700	400	4,700	0	—	—
8	OP	55	×2	800	300	6,700	5,600	500	500
9	InP	11	×2	900	1,000	1,900	5,900	300	100
10	InP	38	×2	0	100	600	100	1,000	2,600
11	OP	12	×3	1,500	0	—	—	100	0
12	InP	40	×3	—	—	300	100	300	400
13	InP	37	×3	—	—	2,900	3,800	700	1,000
14	OP	24	×4	—	—	300	0	0	700
15	OP	16	×5	100	0	900	0	100	100
16	OP	50	×5	1,500	0	2,500	2,800	100	0
17	InP	40	×5	800	0	21,800	400	300	0
18	InP	30	×5	600	0	200	0	3,500	1,100
19	InP	42	×5	—	—	13,400	23,600	0	400
20	InP	50	×5	1,000	100	—	—	300	100
21	InP	40	×5	600	0	100	0	1,500	100
22	InP	51	×5	100	0	13,400	300	—	—
23	InP	12	×5	11,100	6,800	229,800	96,400	0	100
24	InP	45	×5	3,000	2,500	7,400	17,100	600	300
25	InP	12	×5	6,600	11,900	37,400	84,600	2,200	5,400
26	InP	20	×5	300	0	31,800	100	600	500
27	InP	26	×5	100	0	—	—	200	300
28	InP	40	×5	200	0	1,300	0	0	200
29	InP	56	×5	100	0	—	—	200	200
30	InP	50	×5	200	0	15,600	400	200	0
31	InP	48	×5	800	0	391,800	0	700	800
32	InP	15	×5	900	0	10,700	0	500	200
33	InP	16	×5	500	0	109,100	12,300	1,500	12,300
34	InP	13	×5	100	0	5,500	3,200	0	100
35	InP	40	×5	500	0	4,800	0	100	0
Total no. of eggs				43,600	24,100	1,167,400	528,900	19,100	29,400

per gram of feces. On the other hand 4 patients in whose stools no hookworm eggs were observed before treatment had egg counts ranging between 100 and 400 per gram of feces after treatment. The over-all increase in egg counts was 54% (Table 1).

Side reactions. Seventeen of the 35 patients treated showed no unpleasant side reactions. Three patients had nausea alone. Eleven (31%) had both nausea and vomiting. Five of these vomited once, 4 vomited several times and 2 vomited daily during the course of treatment. Four patients had such persistent and severe vomiting that treatment had to be discontinued. Routine blood counts and urine analyses revealed no abnormality before or after treatment.

COMMENTS

Frye *et al.*[1] reported that 87.5% of 16 patients treated with dithiazanine at a dosage of 200 mg

three times a day for 5 days had their trichuris infections eliminated. Swartzwelder *et al*[2] reported 97% cures when 32 individuals with trichuriasis were treated for 5 days at the same dosage. Since then various workers have confirmed the effectiveness of this drug as a trichuricide.[3, 4, 5, 6, 7] Coincidentally while treating for trichuriasis, Swartzwelder *et al*[2] found this drug to have significant activity against *A. lumbricoides*. Of the 42 patients treated, complete elimination of the infection occurred in 28 patients, and the egg count reduction was 97%. The only side reaction observed was vomiting, usually limited to a single episode in less than 20% of the patients treated. Our study shows that in Trichuris infection 16 of the 20 patients treated for 5 days showed no eggs in their stools. In Ascaris infection 7 of the 18 patients treated for 5 days showed no eggs in their stools. These results, though less impressive than those reported by other workers, show significant activity of this drug against these two helminths. However, its activity against hookworm infection is still controversial. Swartzwelder *et al*.[3] reported a total egg-count reduction of 65% in 8 patients with heavy hookworm (*N. americanus*) infection. These patients were treated for 10 days at a dosage of 200 mg of dithiazanine three times daily. These workers also reported that in 23 of 39 patients with hookworm infection treated for either 3 or 5 days at a dosage of 200 mg three times daily, hookworm eggs were not demonstrable by the formalin-ether concentration technique. Wagner *et al*.[4] found that 3 of the 16 patients with hookworm infection had negative stools after treatment. Young *et al*.[5] treated 27 patients with hookworm infection at a dosage of 200 mg four times daily for 5 days. They showed an over-all reduction of hookworm egg counts of 39.6% without being able to eliminate the infection in any of the patients. They also noted occasional increase in the egg counts. Our results appear to show that this drug has no effect against hookworm (*A. duodenale*) infection. The reason for the increase in egg counts of 70% of the 20 patients treated for 5 days is not apparent.

Because of its lack of activity against hookworm and the large number of patients with reactions of nausea or vomiting, or both, dithiazanine appears to be unsuitable for mass treatment of patients in this area where many have multiple infections of *T. trichiura*, *A. lumbricoides*, and *A. duodenale*.

SUMMARY

Thirty-five patients with multiple infections of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale* were treated with dithiazanine iodide at a dosage of 200 mg three times daily for 1, 2, 3, 4, or 4 days.

Significant anthelmintic activity was observed in both trichuriasis and ascariasis but not in hookworm infection. Eighteen of 35 patients had side reactions in the form of nausea or vomiting, or both. In 4 patients the vomiting was so severe and persistent that the treatment had to be discontinued.

Because of its lack of activity against hookworm infection and the large number of patients with side reactions, it is felt that dithiazanine will be unsuitable for mass treatment in this country.

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AMEBIASIS—AN EXPERIMENTAL STUDY

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One objective in studying amebiasis in experimental animals is to evaluate the efficacy of drugs showing anti-amebic properties in vitro before clinical trial. Different species of animals have been employed for this purpose from time to time and their suitability noted. In dogs, for instance, the infection varies in severity unless the animals are kept on salmon diet when the course is more or less uniform and the mortality rate is low enough to permit trial of drugs.[1-3] Kittens are readily susceptible to infection but they do not usually survive the acute attack.[4,5] Macaques being more closely related to man, are expected to be of value but this theory is out-weighed by the fact that macaques may naturally harbor amebae and may not respond clinically when inoculated with *Entamoeba histolytica*.[6-8] In rats, amebic infection usually persists for a short period with spontaneous clearance.[9-11] The evanescent character of infection in this animal weighs heavily against selection of this species.[8] Rabbits appear unsuitable because of the non-abaptability to infection with most strains of *E. histolytica*.[12-15] In guinea pigs, the course is also an acute one and is apt to be fatal.[16-18] Thus none of these animals appears to be an entirely satisfactory substitute for the human host.

Owing to practical difficulties, smaller mammals are preferred, and of the rabbits, guinea pigs and rats, the rats have received more extensive study, especially in experimental chemotherapy. The usual practice generally comprises (a) intracecal inoculation of animals with culture of *E. histolytica* after laparotomy, (b) administration of drugs for a certain period from the date of inoculation, (c) sacrificing all the animals (whether infected or not) after a test period and noting the autopsy findings and (d) comparison of the data with a control group of inoculated but untreated animals. This procedure has its limitations and disadvantages. First, all animals do not usually take the infection from intracecal inoculation with cultures of *E. histolytica* and the onset and course of infection cannot be definitely determined. Second, assessment of the efficacy of a drug by its administration along with the inoculation, *i.e.*, before the amebae have settled in the intestine, irrespective of the fact whether the animals would acquire the infection or not, has obvious difficulty, as there is no scope to study the drug's effect on "relapses" and "chronic state," the major problem in treatment of amebiasis. Third, a large number of animals, whether infected or not, must be sacrificed. Fourth, infection of animals from the inoculation varies even when the animals are kept under identical conditions and consequently the number of infected animals in the treated and control groups may not provide suitable data for comparison.

MATERIALS AND METHODS

Following the technique previously described by us[19] attempts were made to overcome the drawbacks by fixing a discoid hollow silver tube into the cecum, thus establishing a permanent communication between the cecum and the exterior, either on the ventral or dorsal surface of the animal. The procedure was next explored (a) to study the nature of induced amebic infection in guinea pigs, (b) to observe the effect of amebicidal drugs on the acute course, and (c) to follow up the treated animals. Guinea pigs were selected as the host animal in preference to rats because of the availability of space in the cecum to fix the tube and known uniformity of infection by *E. histolytica* inoculation.

Animals were inoculated with a 48-hour crude culture of *E. histolytica* (200,000 parasites approximately per inoculum) through the tube, and the cecal content was aspirated and examined daily for amebae. Routinely three preparations in saline were examined. Usually the cecal content was positive for 1 or 2 days after the inoculation and thereafter became negative. But within the next 3 to 10 days the amebae would reappear in the cecal contents in gradually increasing numbers, the prepatent period thus varying from 4 to 12 days after the date of inoculation. Prior to and during the experiments, the animals' diet

consisted of bran, carrot, beet, lettuce and other green vegetables.

Untreated course. The course of amebic infection as observed in 25 guinea pigs was invariably acute and fatal. The guinea pigs became disinterested in food but had no diarrhea, lost weight and eventually died within a week or 10 days after infection. Autopsy done on 22 animals showed typical amebic ulcers in the cecum in all and scrapings from the ulcers showed numerous trophozoites. The lower part of the colon also showed ulcerated areas, in about a quarter of which amebae could be demonstrated in sections. No obvious change was detected in the liver.

Treated course. In another series of 63 inoculated animals, an amebicidal drug (about 5% of human adult dose) was administered with the onset of infection and the progress assessed by daily examination of cecal contents for amebae. The results are summarized in Table 1.

Failure. Thirty-one animals failed to respond to the treatment and died. On autopsy, typical amebic ulcerations were found in the large intestine, histological sections showing numerous amebae; but the liver did not show any significant change.

Response and recurrence. The animals that responded to treatment and survived the initial infection were followed up. Sixteen of them were specially studied (Figure 1). All showed *E. histolytica* again after varying intervals, simulating a "relapse" for which usually the same drug was repeated and those animals that survived were observed for a further period. They are represented in Figure 2.

It was observed that at the height of infection the animals became listless and looked ill, with disinclination for food and weight loss. But there was no diarrhea. The aspirated cecal contents were often mucoid and contained several amebae. With recovery, the body weight gradually increased but definitely decreased again before reappearance of parasites (? relapse) as will be seen from the following graph (Figure 3).

All 16 guinea pigs were autopsied. Nine showed typical amebic ulcers scattered throughout the large gut. In six, small ulcers were seen in the cecum only and in the remaining one no visible lesion was found.

In accordance with the observations of Carrera and Sadun[20] eleven animals and their livers were separately weighed soon after death. The weight of the liver, the body weight and the percentage which the former is of the latter are tabulated (Table 2).

The weight of the liver of six normal guinea pigs weighing between 280 and 400 grams was also recorded for comparison. The average percentage of liver weight in the fatally infected

TABLE 1

Results from treatment with various drugs

Drugs and dosage	Number of animals	Failure *E. histolytica* persisted and death (days)	Response *E. histolytica* negative on (days)	Relapse *E. histolytica* re-appeared after (weeks)	Death un-explained: stool negative, post-mortem normal
Emetine-bismuth iodide (8 mg daily for 10 days)	7	4 (5–10)	3 (4–7)	2 (2–3)	1
Carbarsone®					
(i) 25 mg daily for 4 days	4	—	4 (2–3)	3 (3–4)	1
(ii) 15 mg daily for 10 days	4	3 (6–10)	1 (4)	1 (4)	—
Kurchi-bismuth iodide (60 mg daily for 10 days)	12	5 (6–12)	7 (2–7)	7 (2–7)	—
Ipecac-bismuth iodide (6 mg daily for 10 days)	12	8 (5–10)	4 (3–8)	2 (4–5)	2
Mebinol V (ethoxy-ethyl-phenoxy-nitro-benzyl-dichloracetamide) (120 mg daily for 10 days)	13	4 (6–9)	9 (2–7)	8 (2–7)	1
Enterovioform (iodoclorohydroxyquinoline) (125 mg daily for 10 days)	7	6 (5–11)	1 (5)	1 (2)	—
Humatin® (Paromomycin) (150 mg daily for 10 days)	4	1 (5)	3 (2–9)	—	—

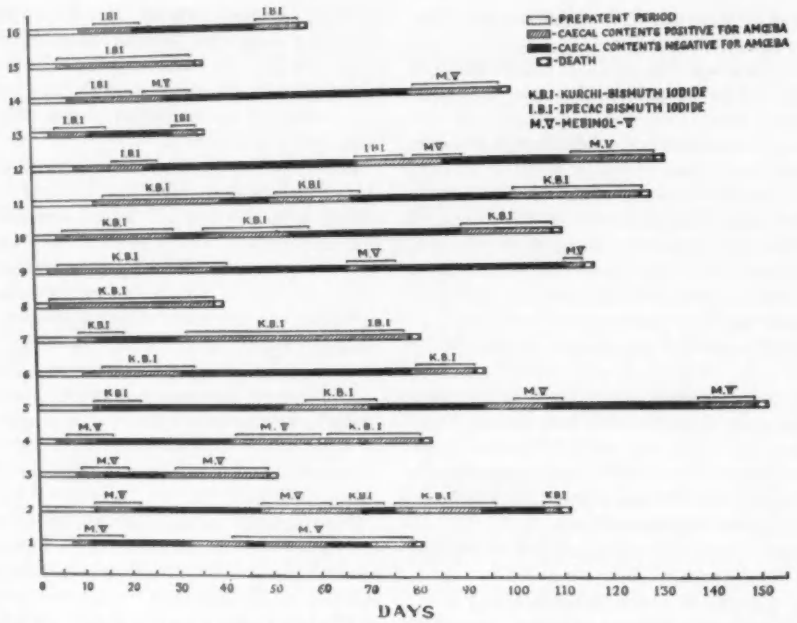

FIG. 1. Course of experimental amebic infection and drug therapy in 16 guinea pigs showing response, recurrence and death.

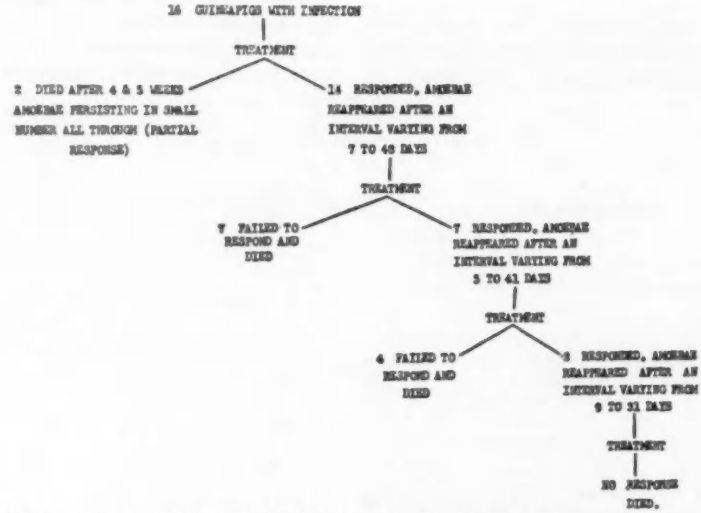

FIG. 2. Schematic presentation of fatalities in 16 guinea pigs with relapse after treatment

and normal animals was 5.01 and 3.58, respectively, of the body weight, indicating definite enlargement of the liver in the infected animals.

Histologically, the sections of the intestine showed numerous trophic forms of amebae lying in the deeper tissue and crypts of Leiberkuhn (Figure 4). The epithelial layer and the basement membrane were disrupted in many areas and

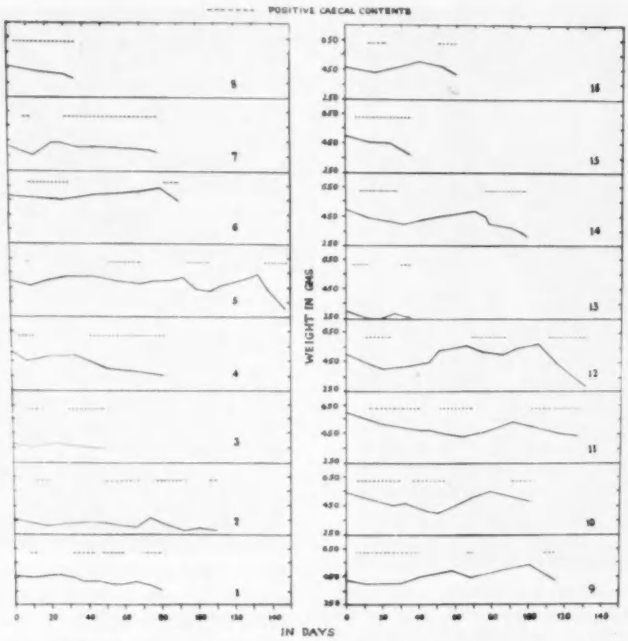

FIG. 3. Graph showing loss of body weight with each recurrence of amebic infection

TABLE 2

Weight of liver in relation to body weight in infected animals

Guinea pigs	Weight of liver (g)	Body weight (g)	Liver weight percentage of body weight
1	19	360	5.27
2	16	283	5.65
4	17	351	4.84
5	17	310	5.48
7	18	380	4.73
9	18	424	4.24
11	20	440	4.54
12	15	271	5.53
14	17	316	5.37
15	19	370	5.13
16	18	416	4.32

the amebae were seen invading the deeper tissues. Occasionally, a large area of the mucous surface along with a part of the submucous layer were necrosed. The deeper tissues beyond the muscularis mucosae were not invaded by amebae. Another characteristic feature was that a large part of the mucous surface was covered by a

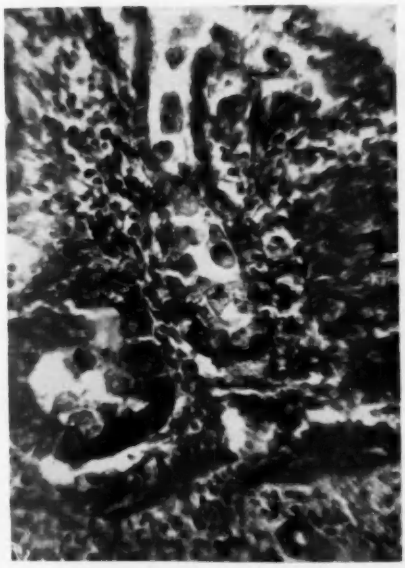

FIG. 4. Photomicrograph showing large number of amebae lying in the crypts of Leiberkuhn, disrupting its wall and invading deeper tissues.

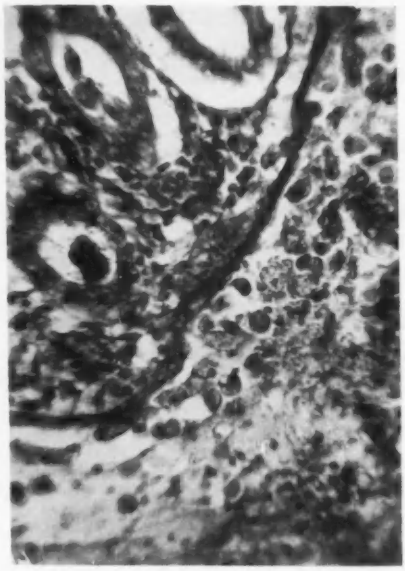

FIG. 5. Photomicrograph showing cluster of amebae in the interspace between the mucous lining of the cecum and the mucinous exudate.

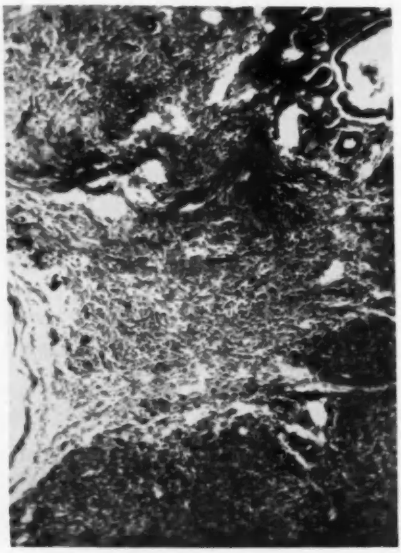

FIG. 6. Photomicrograph showing inflammatory cellular infiltration with fibroblastic proliferation in the portal tracts.

very thick layer of mucinous material and the junctional region contained many amebae (Figure 5). Some of the areas showed infiltration with mononuclear cells and fibroblastic activity indicating an attempt to repair.

The liver in 10 guinea pigs showed moderate fatty change. The portal tracts in seven of them showed accumulation of inflammatory cells, mostly lymphocytes (Figure 6). In three animals the liver sections revealed one or two areas of necrosis with surrounding infiltration of mononuclear cells and occasional polymorphs. No amebae however were detected.

Four control animals fitted with the fixture but not infected were observed for 6 months and then sacrificed; they did not show any abnormality except some local change adjacent to the inserted tube.

Cutaneous ulcer. Three of the "chronically" infected animals while having anti-amebic treatment during "relapse" gradually lost body weight, in spite of the fact that the cecal contents remained negative and the animals were taking food normally. Small ulcers, detected on the abdomen close to the tube, gradually enlarged and were covered with a whitish slough, the

FIG. 7. Cutaneous amebic ulcers around the metallic tube.

margins being red and indurated (Figure 7). Local scrapings showed *E. histolytica* trophozoites. Histological sections of biopsy material from each animal revealed numerous amebae in the dermis without any significant cellular reaction. The three animals were treated locally with the same anti-amebic drugs that were given intra-cecally. Ulcers readily healed and body weight gradually increased. These cutaneous amebic ulcers obviously were produced by local contamination with infected cecal contents.

SUMMARY

A mechanical device, producing a permanent communication between the lumen of the cecum and the exterior, offers a satisfactory method of

studying the course of amebic infection in guinea pigs. Such animals thrive normally with the tube in situ and when inoculated in the cecum with a 48-hour crude culture of *Entamoeba histolytica* acquire the infection within 12 days. The infection takes an acute fatal course without diarrheal manifestations. With early treatment the condition may be controlled, but it usually recurs, simulating chronic intestinal amebiasis, as observed in infected animals kept under observation for several months.

Necropsy showed amebic ulcers in the colon, a large number of amebae being demonstrated in the deeper tissue and crypts. The weight of the liver in relation to the body weight was significantly increased as observed in the relapsing animals as compared to normal ones. Cutaneous amebic ulcers due to local contamination developed in three infected guinea pigs; they were cleared up readily with local anti-amebic treatment.

While illustrating the utility of the mechanical device not only in observing the natural course of amebic infection in guinea pigs but also in assessing the effect of amebicidal drugs on the parasites in the colon, no conclusion was drawn as to the relative efficacy of the different drugs used in the present study.

ACKNOWLEDGMENT

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PROTEIN AND CARBOHYDRATE COMPLEMENT FIXING ANTIGENS OF *TRYPANOSOMA CRUZI**

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Preliminary extraction of *Trypanosoma cruzi* with ether considerably reduced a tendency of saline extracts to fix complement with syphilitic sera,[1] but occasional unexplained reactions continued to be encountered; their frequency varied considerably from lot to lot of trypanosomes, and was approximately the same among healthy as among syphilitic individuals. In attempting to resolve the problem, ether-purified material was fractionated by a method based on the work of Sevag *et al.*[2] This yielded specifically reactive protein and carbohydrate antigens, separable, respectively, in the gel and aqueous phases of chloroform-treated extracts. Both fractions were free of reactivity with normal and syphilitic sera. A description of the procedure follows, with a summary of results obtained in complement fixation tests of sera representing the homologous and several heterologous infections.

MATERIALS AND METHODS

Trypanosoma cruzi. The strain, which was the same as that in the preceding study,[1] was isolated in Brasil (1942) from a case of Chagas' disease. Stock cultures were maintained as previously on diphasic medium consisting of blood agar overlaid with modified Locke's solution. For the mass cultures required in antigen production, however, the organisms were grown in cellulose sacs (*cf.* ref. 3) suspended in blood-coagulum-peptone medium;[4] maximal growth was attained in 30 to 35 days as compared to 12 to 14 days in the previous experience.[1] The original intent of this innovation was to isolate the organisms from solid medium constituents that might contribute to nonspecific reactivity. As events proved, these were not directly responsible; purification of the antigen depended finally on chemical fractionation. However, the cultivation procedure had the important advantage that the trypanosomes at harvest could easily be washed free of medium and metabolites with saline solution. In practice, the cellulose sacs were prepared from 16-inch lengths of $2\frac{3}{32}$-inch Visking casing. After soaking in water, one end of the tube was tied securely in itself, and examined for leaks by filling with water and squeezing. The open end was then tied with string to a 14-inch length of glass tubing (10 mm o.d.), the blind end at the same time being secured in the knot to form a loop; the blind end was punctured just before the knot to vent air. The sac assembly was placed in a 1-liter Erlenmeyer flask containing 20 g of desiccated clots of human blood and 250 ml of peptone solution. The glass tube was left protruding from the flask neck, where it was held with a cotton plug. Fifty ml of peptone solution was introduced through the tube, which was then plugged with cotton. The finished culture flasks were sterilized by autoclaving 20 min at 15 lbs pressure, cooled, incubated overnight at 37°C, and checked for sterility. Each flask was inoculated through the neck tube with about 1 ml of pooled overlay from stock cultures exhibiting satisfactory growth (40 to 50 trypanosomes/hpf), and incubated 30 to 35 days at 23°C.‡ It was then examined for possible contaminants. Pure cultures were harvested by removing sac assemblies from the flasks, rinsing in 0.85% NaCl solution to remove adherent particles, and cutting the sac bottoms to release the contents into a 1-liter beaker. The pooled trypanosomes from all flasks were washed, desiccated under reduced pressure from the frozen state, and stored as in the preceding study.[1]

Antigen fractionation. Desiccated trypanosomes were extracted first with anhydrous ether in the cold,[1] then with veronal-acetate buffered saline solution (VABS),§ using 10 ml of VABS per 100

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‡ Forced-convection bacteriological incubator, refrigerated-heated model #4-489, American Instrument Co., Silver Spring, Maryland.

§ One liter of aqueous solution contained 5.88 g NaCl, 5.09 g $NaC_5H_{11}N_2O_3$, 3.36 g $NaC_2H_3O_2 \cdot 3H_2O$, and 19.2 ml HCl (1 N); the molarity was 0.15, the pH 7.3–7.4.

TABLE 1

Chemical analyses of the antigens

Antigen	Nitrogen (μg/ml)	Phosphorus (μg/ml)	Biuret	Trichloroacetic acid	Molisch
Unfractionated	655	175	Pos.	Pos.	Pos.
Protein fraction	188	11	Pos.	Pos.	Neg.
Carbohydrate fraction	23	8	Neg.	Neg.	Pos.

mg of desiccated organisms; the use of VABS was an innovation but the extraction procedure was otherwise the same as before.[1] For the chloroform-gel fractionation 20 ml of VABS extract was measured into a 4-oz glass bottle with vinylite-lined plastic screw cap. Five ml of chloroform and 2 ml of n-amyl alcohol were added, the bottle capped, and the contents shaken mechanically for 15 minutes at room temperature. The mixture was poured into a 50-ml Pyrex centrifuge tube and spun at room temperature for 5 minutes at 1000 G. The aqueous supernate was transferred by pipette to a clean 4-oz bottle and re-extracted with 5 ml of chloroform and 2 ml of n-amyl alcohol according to the same procedure. Extraction was continued until no gel formed at the chloroform-aqueous interface; 10 to 12 extractions usually sufficed. Tubes containing the chloroform gels were stoppered and stored at 3 to 6°C during the succeeding extractions. Following the last extraction the gels were pooled in a funnel fitted with a Whatman #43 filter paper moistened with distilled water. Aqueous phase entrained in the gels passed through and was combined with the bulk of aqueous extract; this contained the carbohydrate antigen. Gel and free chloroform that remained on the filter were separated by decanting the chloroform. The gel was transferred quantitatively with a rubber "policeman" to a 40-ml graduated, tapered centrifuge tube immersed in a $CaCl_2$-ice water bath (*ca* −18°C). During the following treatments with ethanol and ether, low temperatures were maintained and strict precautions taken to avoid drying the precipitate, which contained the protein antigen. A 15-ml volume of cold (−20°C) anhydrous ethanol was added to the gel, and the precipitate suspended by triturating with a glass rod. The suspension was then spun 15 minutes in the cold (−10°C) at 1000 G, and the supernatant ethanol aspirated through a Richards filter pump. The tube was immediately returned to the $CaCl_2$-ice water bath and the precipitate washed once more by suspending in 15 ml of cold anhydrous ethanol and centrifuging. After the supernate was aspirated, the residue was washed twice more

TABLE 2

Relative specificity of the antigens

Antigen dilution 1:	Reactivity* of the unfractionated (UF), protein (P), and carbohydrate (C) antigens with the given sera															Antigen controls		
	T. cruzi antiserum 1:1			Syphilitic sera 1:1						Normal sera 1:1								
				10657-1			112656-11			41957-1			41957-6					
	UF	P	C	UF	P	C	UF	P	C	UF	P	C	UF	P	C	UF	P	C
1	4	4	4	4	—	—	4	—	—	4	—	—	4	—	—	3	—	—
2	4	4	4	4	—	—	4	—	—	3	—	—	4	—	—	2	—	—
4	4	4	4	4	—	—	4	—	—	2	—	—	3	—	—	—	—	—
8	4	4	4	4	—	—	4	—	—	1	—	—	3	—	—	—	—	—
16	4	4	4	3	—	—	4	—	—	1	—	—	3	—	—	—	—	—
32	4	4	4	2	—	—	4	—	—	1	—	—	2	—	—	—	—	—
64	4	4	4	1	—	—	3	—	—	—	—	—	1	—	—	—	—	—
128	4	4	2	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
256	4	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
512	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1024	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2048	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* The numerical values represent degree of complement fixation, the negative sign (−) no fixation.

with 15-ml portions of anhydrous ether, the material being suspended and sedimented as before in the cold. In aspirating after the second washing, sufficient ether was left to protect the protein from air. Ten ml of chilled (3 to 6°C) distilled water was immediately added, and residual ether removed by passing the tip of an air-exhaust tube over the surface. The protein was dissolved by adjusting the pH to 8.0 with 0.1 N NaOH; solution was assisted with a glass rod. The pH tended to decrease as protein dissolved, and it was necessary to make small additions of NaOH to maintain alkalinity. When solution was complete, distilled water was added to bring the volume to 16 ml.

To remove traces of the organic solvents, both carbohydrate and protein fractions were transferred to cellulose sacs prepared from 8-inch lengths of Visking casing and dialyzed 72 hours at 3 to 6°C against four 250 ml changes of VABS. Each dialysant was then adjusted to 20 ml with VABS, and clarified by spinning 3 minutes in the cold (3°C) at 1000 G. The supernates, constituting the finished antigens, were dispensed in 1-ml volumes in 10-ml Pyrex ampules, shell-frozen, dehydrated under reduced pressure from the frozen state, and sealed hermetically under vacuum; they were stored at −55 to −45°C. For tests the contents of an ampule were rehydrated with 1 ml of distilled water. Antigens preserved in this manner remained stable in activity for at least 5 years.

For the chemical analyses aliquants of ether-purified trypanosome material were extracted in borate buffered saline solution of standard molarity (0.15) and pH (7.3), rather than in the usual VABS, which contained nitrogen. Biuret, trichloroacetic acid, and Molisch tests were conducted according to established procedure.[5] Nitrogen[6] and phosphorus[7] were determined by micro-technics. Analyses of a representative antigen and its fractions appear in Table 1.

Sera. Specimens representing *T. cruzi* or *Leishmania brasiliensis* infections were from the group used in the preceding study;[1] the diagnoses were unequivocally established, and there was no detectable evidence of intercurrent infections. Those representing *L. donovani* infections were from Turkish natives, some of whom had active lesions, whereas others had been clinically cured for the past 2 to 18 years. Specimens from other parasitic diseases had been collected as opportunity arose in cases where the etiologic agent was definitely identified. The syphilitic sera were from individuals with *Treponema pallidum* infections, confirmed either by dark-field demonstration of the treponeme or by specific serologic tests.[8-10] Normal sera were obtained from healthy donors undergoing comprehensive physical examinations as candidates for a service academy; all were negative in a standard cardiolipin slide flocculation test.[11] All sera were stored at −20°C; just prior to use the required volume of thawed serum was heated 30 minutes at 56°C.

Complement fixation. Except for the substitution of triethanolamine buffered saline solution

TABLE 3

Standardization of antigens for complement fixation tests

Antigen	Dilution in TBS 1:	Reactivity* with *T. cruzi* antiserum diluted in non-reactive serum 1:					Antigen controls
		1	2	4	8	16	
Unfractionated	(2)†						
	4	4	3	1	−	−	+
	8	4	4	2	±	−	−
	16	4	4	3	1	−	−
	32	4	4	3	1	−	−
	64‡	4	4	3	1	−	−
	128	4	3	1	±	−	−
Protein fraction	(2)†						
	4	3	1	±	−	−	−
	8	3	2	±	−	−	−
	16	4	2	±	−	−	−
	32	4	2	−	−	−	−
	64‡	4	2	−	−	−	−
	128	4	1	−	−	−	−
Carbohydrate fraction	2	1	1	−	−	−	−
	4	3	1	±	−	−	−
	8‡	4	1	−	−	−	−
	16	2	1	−	−	−	−
	32	1	1	−	−	−	−
	(64)†						
	(128)†						
Serum controls		−	−	−	−	−	−

* The numerical values represent degree of complement fixation, the negative sign (−) no fixation.

† Not tested.

‡ Dilution selected as optimal for diagnostic tests.

TABLE 4

Relative sensitivity and specificity of the antigens in qualitative complement fixation tests of homologous and heterologous sera

Infectious agent or diagnosis	Persons tested	Persons showing the given serologic result* with								
		Unfractionated antigen			Protein antigen			Carbohydrate antigen		
		R	wr	−	R	wr	−	R	wr	−
T. cruzi	40	38	1	1	28	9	3	26	11	3
L. brasiliensis	16	10	5	1	4	2	10	3	2	11
L. tropica	12	2	6	4			12	1	4	7
Schistosomiasis	14	1	5	8			14			14
Filariasis	16	3	5	8			16			16
Trichinosis	4		1	3			4			4
Hookworm	1		1				1			1
Syphilis	49	22	14	13			49			49
Healthy	100	1	7	92			100			100

* Classified as R (reaction = 4+, 3+, or 2+), wr (weak reaction = 1+ or ±), or − (no reaction).

(TBS)[12].* as reagent diluent, the procedure was the same as outlined in the preceding study.[1]

RESULTS

The protein, carbohydrate, and unfractionated antigens were compared with respect to relative specificity by testing serial dilutions of each with reactive syphilitic and normal sera, as well as with specific *T. cruzi* antiserum. Results obtained in a representative assay appear in Table 2; the sera were used undiluted (1:1) to provide as sensitive as possible a test for the corresponding antigen components. While the specific reactivity of the protein (P) and carbohydrate (C) fractions was reduced, as compared to that of unfractionated (UF) antigen, nonspecific reactivity was entirely abolished. The fractions were also free of anticomplementary activity, a characteristic which further differentiated them from parent antigen.

The antigens were standardized for diagnostic complement fixation tests by titrating serial dilutions with similarly diluted homologous antiserum. A typical set of titrations is represented in Table 3. The optimal dilutions were, for unfractionated antigen 1:64, protein antigen 1:64, and for carbohydrate antigen 1:8. The selected dilutions were used in qualitative complement fixation tests of sera representing the homologous, heterologous, and generically unrelated infections. Table 4 indicates the frequency with which specific, cross, and "nonspecific" reactions were encountered in an extended series of comparative tests; sera of healthy individuals were included regularly as controls.

DISCUSSION

A modified chloroform-gel[2] fractionation, applied to ether-purified trypanosomal extract,[1] yielded protein and carbohydrate antigens that were apparently free of nonspecific and anticomplementary activity. The fractions were reduced in sensitivity as compared to unfractionated antigen; this was apparent in titrations with homologous antiserum (Table 3), and in diagnostic tests, where the number of weak reactions was increased (Table 4). On the other hand, both fractions showed a marked compensating improvement in specificity, the borderline reactions (wr) thereby taking on diagnostic significance. Cross reactions with helminthic antisera were eliminated (Table 4), and their frequency in two related leishmanial infections was considerably reduced. The protein antigen in fact did not react

* One liter of stock aqueous solution (concentration 10×) contained 75.0 g NaCl, 1.00 g $MgCl_2 \cdot 6H_2O$, 0.20 g $CaCl_2 \cdot 2H_2O$, 28.0 ml $N(CH_2CH_2OH)_3$, and 180 ml HCl (1 N). Before use 1 volume of stock solution was diluted with 9 volumes of distilled water. The molarity of the diluted solution was 0.15, the pH 7.3–7.4 at 20°C.

with *L. tropica* antisera, and in this respect was superior to the carbohydrate fraction. Of the two purified fractions, the protein appeared to be the antigen of choice for diagnostic tests: it was more sensitive and specific than the carbohydrate, and more economical by virtue of its relatively high optimal dilution (Table 3). The fact that the latter corresponded closely with the optimal dilution for unfractionated antigen suggested that the protein fraction was the major antigen component and that recovery was virtually complete. Direct comparisons with the antigens used in preceding investigations[13-19] would have been of interest, but could not be considered because of material limitations.

Chemical analyses (Table 1) showed that the method of fractionation yielded distinct components; the protein fraction was consistently negative in tests for sugar, and the carbohydrate, despite a low nitrogen content, was uniformly negative in standard tests for protein. In the latter instance, the residual nitrogen may have been due to nonprotein nitrogenous components, and not necessarily indicative of trace protein contaminants. Associated with the fractionation was a marked reduction in total nitrogen, the protein fraction containing 188 μg/ml, as compared to 655 μg/ml for the parent antigen. Ancillary experiments showed the loss to be due largely to dialyzable components. However, these alone did not account for the improvement in specificity, for unfractionated extract could be reduced by dialysis from 655 to 230 μg N/ml without diminishing nonspecific reactivity with syphilitic or normal sera. In view of the relatively low phosphorus content of the protein and carbohydrate fractions (11 and 8 μg/ml) it seemed unlikely that phospholipid was a major component of either antigen.

Following the initial presentation* of this work, attention was drawn to an abstract[20] of an earlier study in which fractions of *T. cruzi* prepared according to Sevag *et al.*[2] had been found to be serologically active. However, the methodology apparently differed somewhat from that presented in the current report. Unfortunately the antigens appear never to have been evaluated with respect to sensitivity and specificity, and comparison with the present work was not possible.

SUMMARY

In attempts to develop a specifically reactive complement fixing antigen, desiccated *Trypanosoma cruzi* rendered essentially lipid-free by extraction with anhydrous ether were extracted in buffered saline solution and the saline extract treated with chloroform and n-amyl alcohol under conditions considered to minimize deterioration. This yielded protein and carbohydrate antigens separable, respectively, in the gel and aqueous phase of extracts thus treated. Each showed a high degree of specific reactivity, as demonstrated in complement fixation tests of sera representing the homologous and several heterologous infections. The findings indicated that the protein fraction was the antigen of choice for diagnostic complement fixation tests.

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THE DISTRIBUTION OF HISTOPLASMIN SENSITIVITY IN GUATEMALA

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During the last 15 years histoplasmin has been widely used in the United States and to a lesser extent in other parts of the world as an aid in defining endemic areas of histoplasmosis. The available information[1] suggests that high rates of histoplasmin sensitivity are found primarily in the Americas with possible areas in Africa and Southeast Asia. However, much of the global information is incomplete because often large geographic areas are represented by data based on relatively small samples from one locale. To our knowledge the only data available for postulating the histoplasmin sensitivity in Central America‡ were obtained from two surveys of sensitivity in the adult population of Honduras[2, 3] and two surveys in children of Nicaragua (Ministry of Public Health, 1950) and of Guatemala.[4] The limited data from Central America and the lack of information on adult histoplasmin sensitivity in Guatemala prompted this study.

The Republic of Guatemala has compressed within its borders a variety of topographical features with elevations ranging from sea level to mountain peaks exceeding 13,000 feet. The flat narrow Pacific coastal plains rise gently from sea level to an elevation of 1,000 feet before erupting into the extensive central highlands. North and east of the continental divide the plateaus, intersected by deep river valleys, gradually slope toward the Caribbean lowlands. Such differences in topography produce distinct physical regions with great differences in climate and rainfall occurring within relatively short distances.

Three overlapping climatic regions are recognized; hot and humid (0 to 3,000 feet), temperate (3,000 to 6,500 feet), and cold (over 6,500 feet). On the highest plateaus (8,000 to 10,000 feet) frost may occur during winter months. In most of the country, the wet season extends from May to November with little or no rainfall during the dry season. Exceptions may be found in the States of Alta Verapaz, in the rain forest of Eastern Petan and in the gulf coastal lowlands, where there is considerable precipitation during the entire year. The annual rainfall ranges from 15 to 20 inches in the arid regions to over 200 inches on the Pacific slope and the eastern rain forest area.

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MATERIALS AND METHODS

Only histoplasmin skin tests were applied in this survey, since a recently completed comprehensive BCG vaccination program precluded the incorporation of simultaneous tuberculin testing.

In February of 1959 the skin testing of 1094 Guatemalan natives was completed by a three member team. An effort was made to obtain information on an equal number of males and females between the ages of 18 and 40 years of age to facilitate the comparison of data from the various test sites. However, the age limitation was not strictly enforced and no person requesting the test was denied.

The attempt to cover widely separated areas during the 3 weeks of the survey necessitated the testing of populations that would remain stable for 48 hours. For this reason, hospitalized patients and some of the attending personnel in 11 government and 2 private hospitals were utilized. Four of these hospitals were located in Guatemala City (Hospital General, Hospital Militar, San Vicente, Hospital Roosevelt), seven were in the provincial towns (Escuintla, Amatitlan, Antigua, Mazatenango, Zacapa, Quezaltenango, Huehuetenango), and two were the private hospitals of the United Fruit Company (Tiquisate and Quirigua). In addition, the skin tests were conducted on soldiers stationed at the military post in Zacapa, and on one occasion during an "open-air" movie for United Fruit Company employees at Quirigua.

Prior to testing, personal data were obtained on each individual including name, age, sex,

hospital ward, bed number and the general area of residence since birth. Clinical charts were also scanned in an effort to eliminate the seriously ill and patients who might have altered sensitivity due to fever or recent trauma. No effort was made to list the racial stock of the subjects (the majority of the patients tested were native Indians and the remainder were of European origin) or their occupations since almost without exception their livelihood was derived directly from the soil.

One-tenth milliliter of 1:100 dilution of histoplasmin (Parke, Davis & Co.) was administered intracutaneously in the right forearm. Forty-eight hours later the transverse diameter of induration was measured with a small flexible ruler graduated in millimeters and recorded. Induration of 5 mm or larger was the criterion of histoplasmin hypersensitivity. All readings were made by the same individual with one exception when the travel schedule required another member of the team to remain at the test site to make the 48-hour readings.

New acid-cleaned 1-ml syringes (60 g of potas-

TABLE 1

Distribution of histoplasmin sensitivity in 821 natives of Guatemala

Test Site	Females		Males		Combined	
	No. tested	% positive	No. tested	% positive	No.	% positive
Quirigua	31	74	55	85	86	81
Escuintla	26	58	31	74	57	67
Tiquisate	15	54	32	71	47	66
Amatitlan	30	53	31	74	61	64
Zacapa*	48	58	68	57	116	58
Mazatenango	33	52	36	61	69	56
Antigua	25	48	50	60	75	56
Quezaltenango	39	51	71	56	110	54
Guatemala†	48	31	99	58	147	49
Huehuetenango	14	14	39	26	53	23
Totals	309	49	512	62	821	57

* Zacapa Hospital, Military Post.

† General Hospital, Roosevelt Hospital, Military Hospital.

FIG. 1. Histoplasmin test sites in Guatemala

sium dichromate in solution with 1,000 ml of concentrated sulfuric acid), and 25-gauge one-half inch needles were used to apply the histoplasmin. Each syringe was usually used for seven consecutive tests while the needles were changed after each skin test dose. Both needles and syringes were reserved exclusively for histoplasmin skin testing.

RESULTS

Forty-eight hour readings of histoplasmin skin tests were made on 1094 of 1241 individuals tested (88%). Included in the 1094 completed tests were 273 proven cases of advanced tuberculosis which will be treated separately.

Positive results were recorded in 468 of the remaining 821 adult subjects. Of these, 315 reactors were in the group of 512 males and 153 reactors were among the 309 female subjects. If one were to compute the mean percentage of

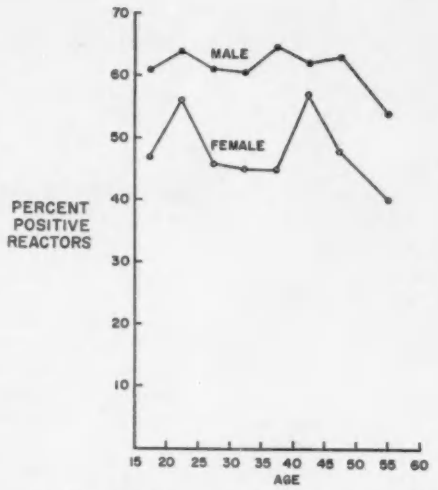

FIG. 2. Histoplasmin sensitivity by age and sex

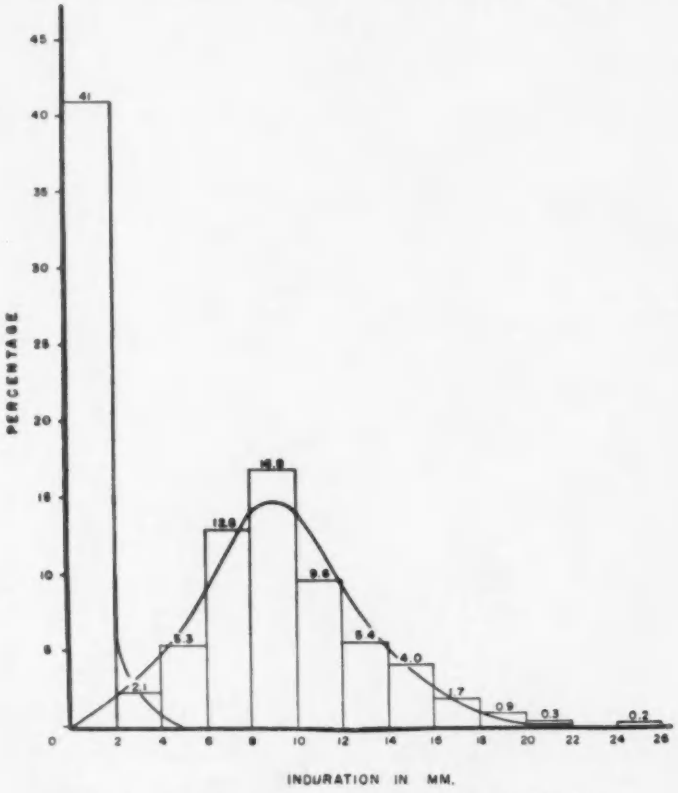

FIG. 3. Frequency distribution of histoplasmin reaction sizes

TABLE 2

Incidence of histoplasmin sensitivity in hospitalized tuberculosis patients

	Number	Number positive	% positive
Females	151	27	17.9
Males	122	25	20.5
Total	273	52	19.1

positive skin tests disregarding geographic area and other factors, the figure would be 57% (468/821). However, in reality the sensitivity ranged from about 23% to 81%.

The distribution of histoplasmin sensitivity is listed in Table 1, in order of descending overall rate. The geographic locations of the 10 communities sampled are indicated in Figure 1. The distribution of sensitivity in the age groups tested (Figure 2) shows only a slight variation in the rates. The youngest person tested was 16 years old and the oldest 60 (only 5 individuals older than 54 years were tested).

The frequency distribution by size of reactions to histoplasmin is presented as a histogram (Figure 3), with the sizes of reactions plotted along the horizontal axis, and the frequency of reactions of specified sizes shown along the vertical axis. This graph illustrates the distribution of reaction sizes in the peoples of Guatemala with the positive reactors (5 mm or greater) forming a curve resembling a normal distribution.

The data obtained on the 273 advanced tuberculosis patients (Table 2) revealed sensitivity in 19%, which indicates a lower incidence of sensitivity in this group than would have been anticipated in the geographic area. One hundred eighty-nine of these patients were hospitalized in San Vicente Hospital (Guatemala City), and the remaining 84 were hospitalized in the Escuintla Hospital. When the observed rate of sensitivity in the patients at San Vicente Hospital (13%) is compared with the rate found in the other residents of Guatemala City (49%), and a similar comparison is made for Escuintla (32% vs. 67%) it appears that over one-half of the tuberculous patients may have displayed altered reactivity to histoplasmin.

DISCUSSION

The 23 to 81% range of histoplasmin sensitivity found in the people of Guatemala revealed localized areas having the highest rate thus far reported from Central America. The previous surveys conducted in neighboring Honduras showed a rate of 41.7% in 300 male hospital patients at La Lima[2] and a rate of 47.7% in 421 hospital patients at Tela.[3]

Inspection of the distribution of sensitivity in our study reveals that the highest incidence of reactors was among the residents of the Caribbean lowlands (Quirigua, 81%), which is characteristically a tropical rain forest belt. The area also has a uniformly humid, warm climate. Conversely the lowest number of reactors was found among the inhabitants of the highest and driest areas (Huehuetenango, 23%).

Plots of histoplasmin reaction frequencies (Figure 3) have been used by other investigators as an index of the specificity of histoplasmin reactions in various geographic areas.[1,5-7] The technique described by Palmer *et al.*[5] was used to draw free-hand curves over the frequency distribution of observed reactions to illustrate the pattern of distribution. The reactions can be seen to fall into a bimodal distribution with a low frequency at 2 to 4 mm. Therefore, the 5-mm criterion used in this study would appear to afford a satisfactory separation of positive from negative reactors. The plot of the reactions measuring 5 mm or greater resembles a normal distribution curve with a maximum frequency at 8 to 10 mm. Similar distributions have been described by Palmer *et al.*[5] and Edwards[6] as characteristic of antigen specific reactions. It is therefore felt that the positive reactors in this survey represent a specific histoplasmin sensitivity rather than cross-reactions or non-specific reactions.

Factors which must be considered as influencing the results in any histoplasmin survey are the ratio of males to females, as well as the age, occupation and race of the test population. The ratio of males to females in this survey is approximately 5 to 3 in the completed tests because many of the women tested were unavailable for the 48 hour readings.

The data presented in Figure 2 illustrate the relatively stable senstivity rate in all age groups of this test population, minimizing the effect of age on the overall results.

The rate observed in the 15 to 19 year age group approximates that found in the 45 to 49 year olds, suggesting that most infections are acquired early in life, and points out the reed for further studies among the child population.

This is substantiated by the low prevalence of reactors (4%) among infants tested in a Guatemala City hospital.[4] Figure 2 also shows the consistently higher percentage of male reactors vs. female reactors in each age group which is a finding consistent with other histoplasmin surveys. Although we, like others before us, considered the assumption that this difference was occupational in origin, we had no opportunity to obtain definitive evidence on this point.

The relative homogeneity of the test population, as described earlier, did not merit analysis of the data on the basis of race or occupation.

SUMMARY

The range of histoplasmin sensitivity in 821 Guatemalan hospital patients between the ages of 15 and 60 years of age was found to be 23 to 81% depending on the test area.

The uniformity of histoplasmin sensitivity rates in all age groups tested suggests that most infections are acquired prior to age 15.

The ratio of males to females was approximately 5 to 3, with sensitivity rates of 62 and 49 per cent, respectively.

ACKNOWLEDGMENTS

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ADENOVIRUSES ISOLATED FROM SAUDI ARABIA

III. Six New Serotypes*

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The first two papers of this series described the epidemiologic features pertaining to 65 strains of adenoviruses isolated in eastern Saudi Arabia and the pathogenicity of three of these strains for man.[1, 2] As might be expected in studies involving virus isolation attempts from hundreds of individuals, six serotypes were encountered which did not fall into the 18 types presently recognized.[3] It is the purpose of this paper to present further data relating to the characteristics of the six serotypes which were reported as untyped in paper I[1] and to suggest that these serotypes be designated types 19 to 24.

MATERIALS AND METHODS

The methods employed for the isolation and identification of the adenoviruses and the various details of tissue culture were reported in the first paper of this series.[1] For neutralization tests, antisera were prepared by inoculating rabbits intravenously several times with the cell sediments of virus pools prepared in a variety of human cell lines, usually human conjunctiva.[4] The rabbits were bled after four or five weekly injections and bled out if the titer was satisfactory; if not, they were given further intravenous booster inoculations. Antisera were prepared against each of the standard types 1 to 18, against strain BAR-2[5] and against each of the six new types. Reciprocal cross neutralization tests were carried out against all the known types and the new types by the routine described by Rowe *et al.*[6] with some minor modifications: one part of virus diluted to produce a definite cytopathogenic effect by the 4th day was mixed with one part of antiserum in a dilution previously calculated by titration in two-fold serum dilutions to completely neutralize the virus prototype for an 8-day observation period. After 30 minutes of incubation at 37°C, 0.2 ml of this mixture was added to each cell culture containing 1.0 ml of medium. Cultures were read blindly at 24-hour intervals for at least 8 days and occasionally for as long as 16 days with some of the slower growing agents. Each neutralization test always included virus plus homologous as well as heterologous antisera; appropriate controls using 1) diluted virus alone, 2) diluted antiserum alone, 3) a mixture of diluted virus plus normal rabbit serum, and 4) uninoculated cultures were invariably employed. Although the tests were ordinarily considered complete on the 4th day after inoculation (Rowe *et al.*[6] read their tests on the 3rd day), the negative cultures were often observed for a further period of 4 days or more after one change of medium. This was found useful for some of the slower growing types such as type 8 or strain 3153, and served as an additional check on the potency of the antisera.

Complement fixation tests utilized whole cell cultures or supernates from such cultures of each virus and known positive adenovirus antisera from a patient who had a severe conjunctivitis due to type 1 adenovirus and from whom both acute and convalescent sera were available. Antigen from uninoculated cell cultures and from cultures inoculated with other viruses such as Coxsackie strains B1 and B2 yielded negative results on numerous occasions. For hemagglutination and hemagglutination inhibition tests the method of Rosen[5] was followed; some of the tests were performed by Dr. Rosen in his laboratory.

RESULTS

Each of the new strains possessed complement fixing antigens common to the other members of the adenovirus group. When each of the prototype strains was used as antigen with acute and convalescent human sera, CF antibody rises from less than 1:10 to 1:80 or greater were demonstrated with each of the viruses.

Under the standard conditions and criteria used in these neutralization tests as described under "Materials and Methods," there was no crossing whatsoever among these six new proto-

* This research was supported in part by the Arabian American Oil Company.

TABLE 1

New adenovirus types from eastern Saudi Arabia

Proposed type designation	Prototype strain	Number of isolations	Reference	Date of collection	Village	Age of patient	Clinical diganosis*
19§	587	4	(1, 9)	3 Aug '55	Khobar	2 yrs	Tr II
20	931	4	(1, 2, 9)	29 Aug '55	Thuqba	7 mos	Follicles & papillary hypertrophy
21	1645	1	(1)	28 Jan '56	Dammam	1.5 yrs	Tr II, F+, P ± V^3, i^3
22	2711	2	(1)	31 May '56	Anek	3 yrs	Tr II, F+, P+
23	2732	2	(1)†	31 May '56	Anek	2 yrs	Tr II, F+, P+
24	3153	1	(1)‡	14 Aug '56	Um es Sahich	1 yr	Tr II, F+, P++. Mild edema of eyelids

* The abbreviations refer to the standard nomenclature for description of clinical findings in trachoma.[10]

† Classified with strain 2711 in reference (1).

‡ Classified as "untyped" in reference (1).

§ This type is indistinguishable from adenovirus type 10 by hemagglutination-inhibition but is a separate type by neutralization tests. (See text.)

types between themselves, between any of the presently described 18 serotypes, or with strain BAR-2.[5] To produce the desired cytopathogenic effect by the 4th day, virus was ordinarily used in dilutions of 1:10 for strains 587, 1645 and 2732; 1:2 for strain 2711; and undiluted for strains 931 and 3153. Antisera which completely neutralized the prototypes for 8 days or longer were used at 1:2 for strains 587, 931 and 1645; 1:4 for strain 2711; 1:8 for strain 3153; and 1:32 for strain 2732. No attempts were made to vary dilutions of virus or antisera to demonstrate possible relationships between serotypes as has been noted with several of the adenovirus types.[3]

Table 1 gives the pertinent details on the new strains. All were isolated from the conjunctivae of Saudi Arab children 3 years of age or younger. So far as could be ascertained from the records, none of the children had ocular disease other than clinical trachoma which affects more than 90% of the inhabitants of this area, including most children in this age group.

Table 2 indicates some of the biological properties noted in the routine pools and passages necessary for the work-up of these strains. Two strains, 2711 and 3153, showed noticeably slow growth in tissue culture with incomplete cytopathogenic effects even when held for long periods of time. On the other hand, strains 587 and 1645 grew rapidly to high infective titer and the cytopathogenic effect quickly involved the entire culture. As has been found by others[7] there was no relation between the infective titer and the ability of the strains to elicit specific neutralizing antibodies in the rabbit; several attempts to produce antisera in titers higher than 1:32 against strain 587 and the three strains identical to it have so far proved unsuccessful. Table 3 gives the pattern of hemagglutination obtained

TABLE 2

Some properties of adenovirus types 19–24 from Saudi Arabia

Proposed type	Strain	Highest infective titer in cell culture TCD_{50} per ml (log 10)	Homologous titer of rabbit antiserum (reciprocal)	Growth characteristics
19†	587	8.0	32	rapid growth, complete CPE*
20	931	5.7	>16	moderate growth, incomplete CPE
21	1645	7.7	>4	rapid growth, complete CPE
22	2711	4.7	>128	slow growth, incomplete CPE
23	2732	5.7	128	moderate growth, incomplete CPE
24	3153	3.7	>128	very slow growth, incomplete CPE

* CPE = cytopathogenic effect.

† See footnote, Table 1.

TABLE 3

Hemagglutination characteristics of adenovirus types 19–24 from Saudi Arabia using erythrocytes of three species (reciprocal of HA titers)

Proposed type	Strain	Human "O"	Rhesus	Rat
19*	587	160	—†	160
20	931	—	—	—
21	1645	—	160	—
22	2711	—	—	2560
23	2732	—	—	2560
24	3153	—	—	2560

* See footnote, Table 1.

† (—) indicates no hemagglutination.

from these virus types. No attempt was made to examine this aspect of the virus types in detail, but it is noteworthy that hemagglutination titers appeared to have no close relation to infective titers in cell culture. In the case of strain 931 which did not yield hemagglutinin in several different tests, the infective titer of the pools was checked and shown to contain more than 10^7 TCD_{50}'s per ml. This then would compare with Rosen's findings that types 12 and 18 did not yield hemagglutinin.[5]

It is pertinent to describe the conditions surrounding the isolations of strains 2711 and 2732. On May 31, 1956 two members of the Aramco trachoma research unit surveyed a group of children ranging in age from 45 days to 9 years in the village of Anek. This village is deserted in the winter grazing season and the Bedou population had just arrived from the desert. Of 42 children examined, 38 had various stages of active trachoma, although no acute conjunctivitis was noted. From 9 children with the most active trachoma a conjunctival scraping was taken by methods previously described,[1] and two adenovirus strains, 2711 and 2732, were later isolated from this group. The two children came from different families. Another isolation of strain 2711 was made from a permanent resident of a village several miles away in September, 1956, while a second isolation of strain 2732 was obtained from a different Bedou settlement on June 24, 1956.

DISCUSSION

As previously reported[1] these new strains were isolated sporadically; only one isolation was obtained of strains 1645 and 3153, two of strains 2711 and 2732, and four of strains 587 and 931. This contrasts with 10 isolations of type 8, ten of strain BAR-2, eight of type 3, and eight of type 17; thus these four types account for 55% of the total of 65 adenovirus strain isolations in Arabia. Under the field conditions as previously described[1] it was the purpose of the Aramco research team to survey various populations for pathogens of the eye; it is not possible to state whether or not these agents were the cause of disease in Arabia or whether they were present in epidemic form. The only strain which has been tried in volunteers is strain 931 which produced conjunctivitis and folliculosis.[2] From a biological point of view these new serotypes are unremarkable and resemble other serotypes previously reported.[3] Strains 587 and 1645 are similar to other rapidly growing types such as types 1 or 7. Strains 931, 2711 and 2732 grew more slowly and, resembling type 4 or 6, had lower infectious titers, while strain 3153 grew very slowly and seemed to have biological characteristics more similar to those of type 8.

A certain amount of crossing in hemagglutination inhibition tests was observed with these virus strains. Rosen (personal communication) noted that strain 587 was identical to type 10 by hemagglutination inhibition tests, but differed by neutralization. No crossing in neutralization tests was observed in our laboratory between type 10 and any of four separate isolations of strain 587. We observed some crossing in the hemagglutination inhibition test between strains 2711 and 2732, but no crossing whatsoever was noted in repeated neutralization tests both in our laboratory and in Rosen's. Since Rosen[6] has found significant cross reactions in hemagglutination inhibition tests between a number of serotypes, for example between types 7, 11 and 14 and between types 8 and 9, the above cross reactions were not unexpected and the final interpretation as to typing was based solely on neutralization tests.

SUMMARY

Six new adenovirus serotypes are proposed. The prototypes were encountered among 65 strains of adenoviruses which were isolated from eastern Saudi Arabia in the period 1955 to 1957. Suitable prototype materials have been forwarded to the subcommittee on adenoviruses of the National Institutes of Health.

ACKNOWLEDGMENTS

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THE ACTION OF SUNLIGHT ON SODIUM PENTACHLOROPHENATE

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It has been recognized that photochemical degradation of sodium pentachlorophenate (NaPCP) may be a factor which reduces the efficacy of this compound as a molluscicide in streams or ponds exposed to bright sunlight.[1,2] No instance in which sunlight appreciably affected the performance of this agent in the field was reported, however, until R. J. Pitchford and his associates (personal communication) noticed that in certain streams in South Africa the use of NaPCP gave unexpectedly poor control of the snail vectors of schistosomiasis. The failures occurred in small shallow streams which flowed rapidly and lay in full sunlight during the day. The water in these streams, although very hard (up to 650 ppm $CaCO_3$), was exceptionally clear. Pitchford speculated that the NaPCP was being destroyed by sunlight and that the greater success experienced with NaPCP in other geographical areas was at least partly attributable to conditions which protected this compound from the destructive action of sunlight.

Meyling *et al.*[3] conducted a field study of the use of NaPCP in the Transvaal and concluded that light was the most important single factor affecting the deterioration of NaPCP under the conditions prevailing in that area. These findings interested us in undertaking laboratory experiments to learn more about the effect of light upon NaPCP and to establish a basis for appraising the practical significance of this reaction.

MATERIALS AND METHODS

Stock solution of sodium pentachlorophenate. A concentrated stock solution was prepared by dissolving 10.0 mg of pentachlorophenol, C.P., (Eastman) in 5 ml of saturated $NaHCO_3$ solution by warming on a steam bath. The solution was cooled to room temperature and diluted with distilled water to 100 ml. This stock solution had a pH of 8.5 and was stable for several weeks or more when stored in the dark at 4°C. Solutions of NaPCP used in the irradiation experiments were prepared by further dilution with distilled water. Solutions to which snails were later exposed were prepared as indicated except that tap water known to be suitable for the snails was used instead of distilled water.

Chemical assay of NaPCP. Test solutions of NaPCP were analyzed before and after irradiation by removing duplicate 1-ml aliquots, diluting each to 5 ml, and adding 1 ml of standardized methylene blue reagent.[4] Each sample was then extracted with 5 ml of chloroform and a suitable volume of the chloroform phase was removed to a 1-ml cuvette for determination of optical density at 655 mμ in a Beckman DU Spectrophotometer. The blank consisted of a similar chloroform extract of a 5 ml sample of the distilled or tap water used in the experiment.

Snail egg tests. Samples of NaPCP solutions before and after exposure to light were tested for their ability to kill eggs of *Australorbis glabratus*. Intact clutches of eggs were collected on thin plastic sheeting.[5] The clutches contained 10 to 48 eggs each and were used within 1 day after laying. The eggs were added to specimens of the solutions to be tested which were then maintained at room temperature for 24 hours. At the end of this time the number of dead eggs could be determined by direct count.

RESULTS

Photochemical experiments. The absorption spectrum of an aqueous solution of NaPCP, shown in Figure 1, has major peaks at 245 and 318 mμ. Only the higher of these wavelength regions is of interest in connection with the stability of NaPCP in sunlight, since the amount of light reaching the earth's surface from the sun is negligible at wavelengths less than 290 mμ.[6] To provide a source of illumination in this region a General Electric RS (275 watt) sunlamp was used at a distance of 10 cm above the surface of the liquid, which was contained in 5-ml beakers. A 1-mm thickness of Pyrex glass covering each beaker excluded essentially all light of wavelength less than 290 mμ. Some specimens, in

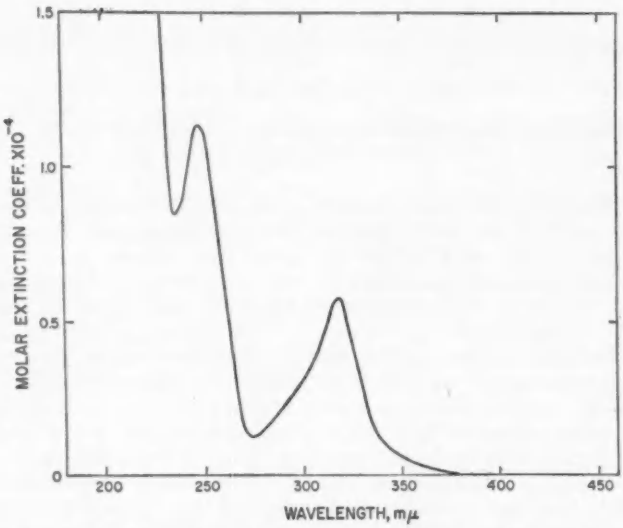

FIG. 1. Ultraviolet absorption spectrum for sodium pentachlorophenate in dilute aqueous solution at pH 7.

addition, were covered by 4 mm of soft glass which cut off all wavelengths below 330 mμ.

When 3-ml aliquots of a solution containing 10 ppm of NaPCP were irradiated for varying periods of time, and each aliquot was assayed colorimetrically, the results shown in Table 1 were obtained. It can be seen that the specimens under the soft glass filters were not affected by the light, indicating that the wavelengths above 330 mμ were not contributory. The loss of NaPCP in the other specimens therefore resulted from absorption of light in the wavelength region of 290 to 330 mμ, corresponding approximately to the 318-mμ peak in the absorption spectrum. Mean intensity of the effective light was estimated to be 0.04 watts/cm² on the basis of photocell measurements.

TABLE 1

Photochemical degradation of sodium pentachlorophenate (NaPCP) solution (initial concentration 10 ppm)

Filter	Time (min)	Concentration (ppm)	$\frac{c}{c_0}$*
Pyrex (excludes light of wavelength less than 290 mμ)	0	10.0	1.00
	30	6.5	0.65
	60	3.5	0.35
	90	2.5	0.25
	120	1.5	0.15
Soft glass (excludes light of wavelength less than 330 mμ	0	10.0	1.00
	30	10.0	1.00
	60	10.0	1.00

* $\frac{c}{c_0}$ is the fraction of NaPCP remaining as determined by colorimetric analysis.

The degradation of NaPCP, as measured by the colorimetric method, followed first-order kinetics, since $-\log \frac{c}{c_0}$ was a linear function of time of irradiation, as shown in Figure 2.

Tests for molluscicidal activity of solutions exposed to light. Flat Pyrex crystallization dishes each containing about 1 liter of dilute NaPCP solution, in a layer 2-cm thick, were exposed to sunlight for a time sufficient to reduce the NaPCP concentration (as determined by chemical assay) by two-thirds. The irradiated samples, and corresponding unirradiated controls, were analyzed and diluted so that test and control samples contained equal amounts of NaPCP as determined chemically.

The percentages of snail eggs killed in 24 hours by various concentrations of irradiated and control solutions in two experiments are shown in

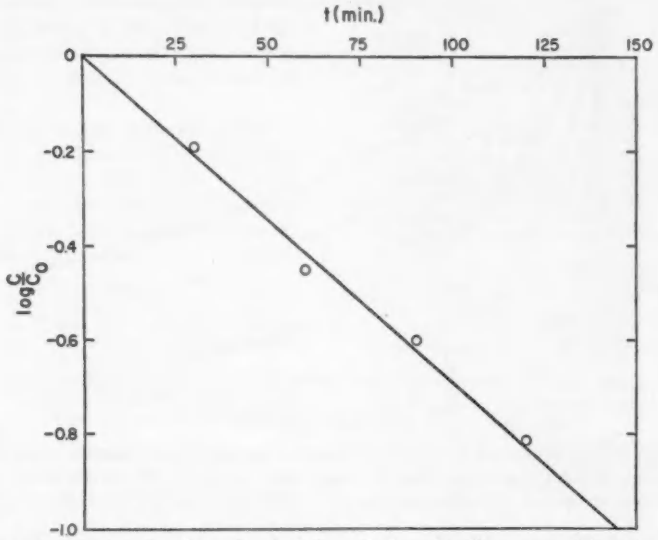

FIG. 2. First-order plot for the photochemical degradation of sodium pentachlorophenate (10 ppm) in aqueous solution. Velocity constant was 3.4×10^{-4} sec^{-1} at a light intensity of approximately 0.04 watts/cm^2 between 290 and 330 mμ.

TABLE 2

*Percentages of snail eggs killed within 24 hours by irradiated and untreated solutions when each solution was diluted to the same NaPCP concentration**

	Test 1			Test 2		
	ppm NaPCP			ppm NaPCP		
	1.0	0.5	0.25	0.34	0.20	0.12
Irradiated solution						
No. of eggs	116	160	182	90	66	68
Per cent dead	98	89	88	92	73	0
Untreated solution						
No. of eggs	128	107	130	74	80	75
Per cent dead	100	100	82	100	76	5

* Average loss among water controls was 2%.

Table 2. The lethal effects of irradiated and control solutions were not significantly different. This indicates that the decrease in NaPCP concentration in the irradiated samples, as measured by the chemical method, was paralleled by a decline in molluscicidal activity.

DISCUSSION

That sodium pentachlorophenate should undergo a chemical change upon exposure to bright light is not surprising in view of the general instability of substituted phenols. It is of considerable interest, however, that the ability to render methylene blue soluble in chloroform and the ability to kill snail eggs are lost concurrently. It may be concluded that the products of the photochemical reaction neither influence the solubility of methylene blue, nor have any appreciable activity against snail eggs. The reaction of NaPCP to light, which may be relatively complex, possibly involves oxidation of the pentachlorophenol to the corresponding semiquinone as a first step following the usual route of degradation for this type of compound.[7]

In appraising the significance of these experimental observations in terms of the field use of NaPCP, it is necessary to make an initial assumption about the intensity and duration of the sunlight to which the treated bodies of water are likely to be exposed. From the data of Luckiesh[8] who tabulated intensity as a function of wavelength for a horizontal surface in direct sunlight at noon on a clear midsummer day in Cleveland, Ohio, it may be calculated that an

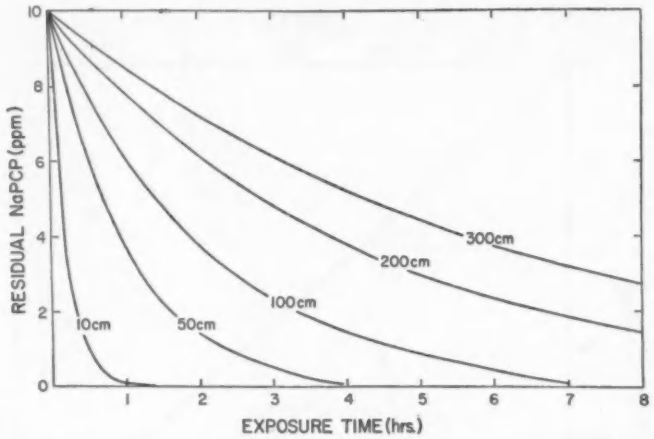

FIG. 3. Predicted photochemical destruction of sodium pentachlorophenate in clear, flowing streams of various depths. Results were calculated for continuous exposure to sunlight of constant intensity equivalent to that prevailing at noontime on a clear midsummer day in Cleveland, Ohio.

incident light intensity of 0.56 watts/cm² over the wavelength range of 290 to 330 mμ would be provided by illumination from the sun under these arbitrary conditions. Geographic, seasonal, and diurnal variations in sunlight intensity are so great that this value can offer only a very rough approximation of the effective incident intensity one might encounter under field conditions.

The fraction of NaPCP remaining at time "t" in water exposed to sunlight may be calculated[9,10] as

$$\frac{c}{c_0} = e^{-\frac{kI_0t(1-e^{-\alpha L})}{\alpha L}}$$

for a body of water which is stirred by turbulent flow or convection. In this equation, "k" is the velocity constant, "I_0" the assumed incident light intensity, "α" the absorption coefficient, and "L" the depth of the water. Substituting observed values for "k," and "α," assuming "I_0" to be 0.56 watts/cm² and "c_0" to be 10 ppm, and calculating "c" for various values of "L" leads to the set of curves shown in Figure 3. These curves illustrate the effect of depth of water upon the persistence of active NaPCP. It is evident that NaPCP is much more likely to be vitiated by photochemical action when added to very shallow streams or ditches than when added to deeper bodies of water. Moreover, it is apparent that under certain conditions the destruction of NaPCP by light may be very rapid.

Clarity of the water is also a factor of great importance since (as shown in equation 1) absorbency and depth of the liquid are complementary parameters, *i.e.*, doubling the absorbency of the liquid would have the same effect as doubling the depth as far as the course of the reaction is concerned. It is evident that, in clear, shallow water, NaPCP is rapidly destroyed by the action of sunlight. On the other hand, turbid water, such as that encountered in many streams and irrigation ditches, would permit only limited penetration by light, and NaPCP under these conditions would be relatively well protected against photochemical action. The favorable influence of turbidity may have contributed materially to the success of NaPCP when used against snails in Egypt[11] and other places.

It may be concluded that, other things being equal, NaPCP will persist longer in bodies of water which are shielded from direct sunlight by trees or other vegetation, and it also appears that NaPCP treatment of exposed bodies of clear water would be more successful if done during the night.

SUMMARY

Irradiation of dilute aqueous solutions of sodium pentachlorophenate (NaPCP) with light in the wavelength range of 290 to 330

mμ causes a chemical alteration of the NaPCP with a concomitant loss of molluscicidal activity. The reaction follows first-order kinetics, and the velocity constant is directly proportional to light intensity.

When NaPCP is used to treat the water of shallow ponds or streams exposed to bright sunlight, photochemical degradation of this agent may be an important variable, especially when the water is clear and free of solutes which interfere with light penetration.

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STABILITY OF PRIMAQUINE DIPHOSPHATE UNDER VARIOUS CONDITIONS

LEO E. GAUDETTE* AND G. ROBERT COATNEY

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Parasite Chemotherapy, Bethesda, Maryland

In certain areas of the world, malaria eradication can be achieved by the use of residual insecticides. In other areas, however, because of the habits of the vector, mores of the people, and logistic problems, other methods must be employed if eradication is to be achieved. The desired results might be obtained by means of mass chemotherapy, but there is always the the problem of getting drugs to the people. This would be a simple matter if an effective and safe drug were combined with a universal article of the diet. In 1954, Pinotti[1] showed that chloroquine was effective when combined with table (cooking) salt, and Coatney *et al.*[2] confirmed Pinotti's results with chloroquine, and showed that pyrimethamine was equally effective under the same conditions. Each of these drugs was stable and safe under conditions employed in the preparation of food, and each is an excellent suppressive, *i.e.*, the action is directed primarily against the parasites in the circulating blood. Primaquine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline, on the other hand will destroy tissue stages of the parasite responsible for relapse[3] and is also highly effective against gametocytes.[4] If this drug were stable under conditions encountered in the preparation of food, it might be extremely useful in a malaria eradication program. This report presents the results of a study of primaquine stability.

MATERIALS AND METHODS

Estimation of Primaquine†

The ultraviolet absorption characteristics of a solution of primaquine in 0.1N H_2SO_4 were determined in the Beckman DU Spectrophotometer. Primaquine has a characteristic curve with maxima at 224, 266, 282 and 330 mμ; and minima at 216, 250, 276 and 310 mμ. A primaquine solution of 10 μg/ml had an optical density of 0.375 at 282 mμ. Optical densities were proportional to concentrations.

Primaquine was isolated from test preparations at alkaline pH by extraction into ethylene chloride. The compound was then returned to an aqueous phase by shaking with 0.1N H_2SO_4. By this method, recoveries of more than 90% were obtained at concentrations of 10 to 200 μg/ml.

Stability Tests

Conditions for testing the stability of the compound simulating those met with in the cooking of foods were as follows:

Dry heat test in presence of salt. A 25-milligram portion of primaquine diphosphate and 5.0 g of NaCl were intimately mixed by grinding. This mixture was placed in an oven at 100°C for 24 hours. The mixture was thereafter dissolved in water, diluted to 500 ml and assayed for primaquine by the procedure described. The optical density of this solution was compared with a standard treated in exactly the same way but not submitted to heat treatment.

Boiling water test. A 1-gram portion of primaquine diphosphate was dissolved in 100 ml of water and refluxed for 24 hours. At the end of the test period, a sample of the material was diluted to the theoretical concentration of 20 μg/ml, extracted as described and assayed by its absorption at 282 mμ. A fresh standard prepared in exactly the same way was employed for comparison.

Oil heat test. Two 0.25-gram portions of primaquine diphosphate were placed in two test tubes, and 3 g of melted hydrogenated vegetable oil (Crisco) was added. The solid was suspended in the oil by stirring. One tube was heated in an oven at 100°C for 24 hours; the other was placed in a mineral oil bath at 200°C for 1 hour. At the

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† Primaquine diphosphate was obtained from Lilly Research Laboratory, #086410, Lot 021–97–257.

end of the respective time periods, 10N NaOH was added and the material extracted and assayed as previously described. Suitable standards were prepared for comparison.

Antimalarial activity of test samples. The antimalarial activity of the material in each test sample was determined by administering the equivalent of the maximum tolerated dose, 7 mg of primaquine diphosphate per kg,[5] once daily for 4 days to young chicks infected with *Plasmodium gallinaceum*. The chicks were infected by the intravenous inoculation of 16 million parasitized erythrocytes on the day preceding the 1st day of treatment. The test preparations were administered orally by tube. Blood films were taken from all test animals on day 4 and 5, and stained with Giemsa. Parasite estimations were on the basis of counts per 10,000 red blood cells.

RESULTS

A visible change in the appearance of each test sample was observed almost immediately after initiating the heat treatment. The water solution of primaquine was changed from straw-colored to dark red; the salt preparation and the oil-suspended materials similarly darkened. In addition, in the mineral oil bath preparation at 200°C, a dark tar-like material formed on the sides of the tube.

TABLE 1

Primaquine diphosphate stability under conditions simulating those in the preparation of foods

Test condition	Time hr	Primaquine concentration		Recovery per cent
		Before	After	
		μg/ml	μg/ml	
Oil (100°C)	24	50	47	95
Dry heat (100°C)	24	50	35	70
Boiling water	24	20	9	47
Oil heat (200°C)	1	50	3	6

The primaquine content of the test samples

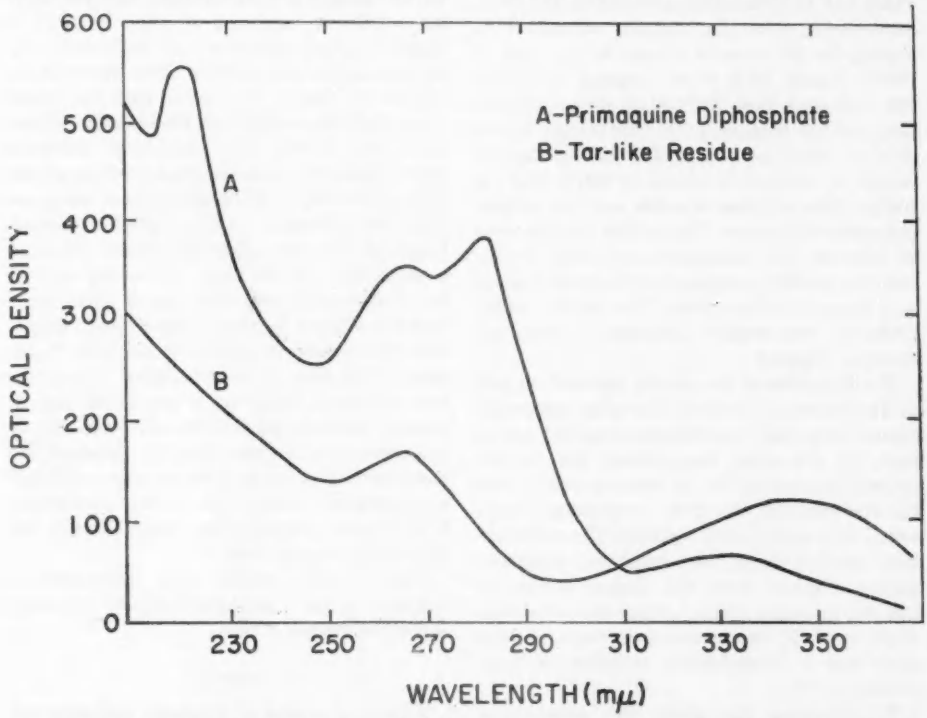

FIG. 1. Absorption spectrum of primaquine diphosphate and of the tar-like product obtained by heating 250 mg of primaquine diphosphate in 3 grams of hydrogenated vegetable oil (Crisco) at 200°C for 1 hour.

TABLE 2

Antimalarial activity of heat-treated primaquine diphosphate

Test condition (primaquine)	Number of animals	Average weight (grams)	Parasite count	
			Day 4	Day 5
Control animals....	5	58	1700	5300
Fresh solution.....	5	66	2	0
Dry heat (100°C)...	5	56	886	4860‡
Boiled water.....	5	57	60*	36*
Oil heat (200°C)†...	5	55	1218	4410‡

* Atypical parasites and host cells.

† The 100°C oil heat sample was lost prior to these tests.

‡ Gametocytes present.

was determined by extraction and spectrophotometric assay. The results are summarized in Table 1. Varying amounts were recovered as apparent primaquine from each test preparation. There was an apparent recovery of 95 and 70%, respectively, from the samples submitted to heating for 24 hours in oil and in dry heat at 100°C. Nearly 50% of the original compound was recovered from the boiling water preparation, and less than 10% from the sample heated in oil at 200°C for 1 hour. The tar-like material formed in the sample heated to 200°C was extracted into ethylene chloride and the extract concentrated *in vacuo*. The residue was dissolved in ethanol. The absorption spectrum of this material was then compared with a fresh solution of primaquine diphosphate. The results, which produced two totally dissimilar spectra, are shown in Figure 1.

The formation of this tar-like material, as well as the deepening in color of the other test preparations suggested a condensation reaction due to heat. At the lower temperature, the original material seemed stable. It was conceivable that the recoveries at the lower temperature represented only an apparent stability. The condensation reaction being less extensive, molecular species, different from the original compound but still retaining similar optical characteristics, might exist. It was necessary to learn whether there was a corresponding retention of antimalarial activity.

To determine this point, test preparations were administered at the maximum tolerated dose to young chicks infected with *P. gallinaceum*. The results are summarized in Table 2. In contrast to the stability data, the antimalarial activity of these preparations was destroyed. The boiled water preparation was an exception for it retained some of its antimalarial activity. A similar infection might be anticipated if primaquine were administered at the smaller dose of 0.5 mg/kg/day. Small numbers of parasites appeared in blood of chicks receiving the boiled water preparation, but the parasites and their host cells were atypical. The changes which the primaquine undergoes are apparently even more extensive than the spectrophotometric assay procedure would indicate.

DISCUSSION

The results of chemical analysis indicated varying degrees of instability of primaquine diphosphate under different conditions of heat treatment. As a result of the formation of a polymer-like material at high temperature, and visible changes in color intensity with the varying conditions employed in other tests, it is suggested that alteration of primaquine by polymerization occurs. Since there were varying degrees of change, it appears that the extent of condensation varied with the intensity of heat treatment. If this were true, small polymers might retain the natural characteristics of the primaquine nucleus and might be read, under our analytical procedure, as the original compound. Consequently, the analytical results obtained could actually be too high. This would seem to be in agreement with the loss of antimalarial activity. When a portion of the treated material was administered to chicks infected with *P. gallinaceum* at doses based on original primaquine base known to be active, it was found that, in general, antimalarial activity had been lost. In one instance where some activity remained, the parasitemia was low and the parasites exhibited morphological changes. The chicks died within 5 days after stopping the drug although the parasitemia was still low.

These results indicate that primaquine is unstable under conditions normally occurring in the preparation of food.

SUMMARY

When primaquine diphosphate was subjected to conditions simulating those ordinarily occurring in the preparation of food, it was found to

be unstable. Spectrophotometric analysis indicated a destruction of from 5% to 94% depending upon the conditions of heating. However, when the heated samples were tested against *Plasmodium gallinaceum* in young chicks, all activity was absent except in the sample boiled in water for 24 hours and in this instance the activity was of a very low order. These findings exclude the use of primaquine as a salt additive in malaria eradication programs.

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Amsterdam, March 7, 1960

To the Editor.

Sir,

Considering the interesting piece of work by Varela, Kean, Barrett and Keegan on the important problem of diarrhea among travelers, which has been published in your Journal (1959, **8:** 353–357), it seems that the results of this investigation deserve more attention than the seemingly disappointing conclusion, stating that the enteropathogenic *E. coli*, *Paracolobactrum* and *Klebsiella* could not be incriminated as the cause of the diarrhea of travelers to Mexico.

Among the 27 students, who contracted diarrhea, bacterial pathogens were significantly more often isolated than among the 35 well students. The probability that the preponderance of pathogens among the sick students could be due to mere chance is less than 5% ($\chi^2 = 5.0407$; one degree of freedom).

More important, however, is the observation that the occurrence of isolated bacterial pathogens among the total group of 62 students is not equally distributed during the period of study. After rearranging the data, given in Tables 1 and 2 of Varela's publication, according to the number of days after arrival of the students in Mexico (see Table 1 or Figure 1), it becomes evident that pathogenic *E. coli* was exclusively isolated within the first 9 days after arrival, whereas *Paracolobactrum and Klebsiella* were predominantly cultured during the subsequent period of investigation. This provides more than suggestive evidence that these particular microorganisms should be considered as true invaders, rather than as normal intestinal inhabitants.

This gets further support when one considers the occurrence of diarrhea during the period of study. Not a single case of diarrhea was mentioned on the 11th day after arrival, whereas, in the period before, 19 episodes of diarrhea with 42 diarrhea days were reported, and from the 12th day onward 14 episodes of diarrhea with a total of 30 days. The reporting of diarrhea clearly shows a bimodal distribution during the period of observation. There is no reason to suspect that the students failed to report around the 11th day, because fecal specimens were submitted as usual. More likely is the assumption that two waves of diarrhea occurred. The first one appeared within 10 days after arrival and seems associated with the invasion by pathogenic *E. coli*. The occurrence of diarrhea in the second period is readily explained as a result of the infection with *Paracolobactrum* and/or *Klebsiella*.

TABLE 1

Occurrence of diarrhea and isolation of enteric bacteria among newly arrived students in Mexico, as shown by rearrangement of data reported by Varela et al. *(1959)*, Am. J. Trop. Med. & Hyg., **8:** *353–357*

Number of days after arrival in Mexico	Number of persons examined on each day	Number of isolations			Number of students reporting a new episode of diarrhea*	Number of students reporting diarrhea
		Pathogenic *E. coli*	*Paracolobactrum*	*Klebsiella*		
1	9	—	—	—	—	—
2	34	—	—	—	—	—
3	49	2	—	—	—	—
4	53	1	—	2	1	1
5	55	1	1	1	3	3
6	55	1	—	—	3	6
7	56	1	1	—	4	7
8	56 (55)	1	1	1	4	10
9	60 (59)	4	2	—	3	9
10	60	—	2	—	1	6
11	62	—	9	1	—	—
12	62	—	6	1	5	5
13	62	—	4	3	2	5
14	62	—	3	1	4	6
15	53	—	1	2	–	5
16	28	—	5	1	2	6
17	13	—	—	2	—	2
18	9	—	1	—	—	—
19	7	—	1	—	—	—
20	7	—	1	—	—	—
21–26	(16)	—	—	—	1	1

* The recurrence of diarrhea after an interval of more than 1 day was considered as a new episode of diarrhea.

In this respect it is not surprising to note that the enteric bacteria were isolated among sick as well as among healthy students. Susceptible persons may respond to the invasion of enteric pathogens with diarrhea, whereas other persons with a higher specific or non-specific resistance (in other words, with a well-trained intestinal tract) may remain healthy.

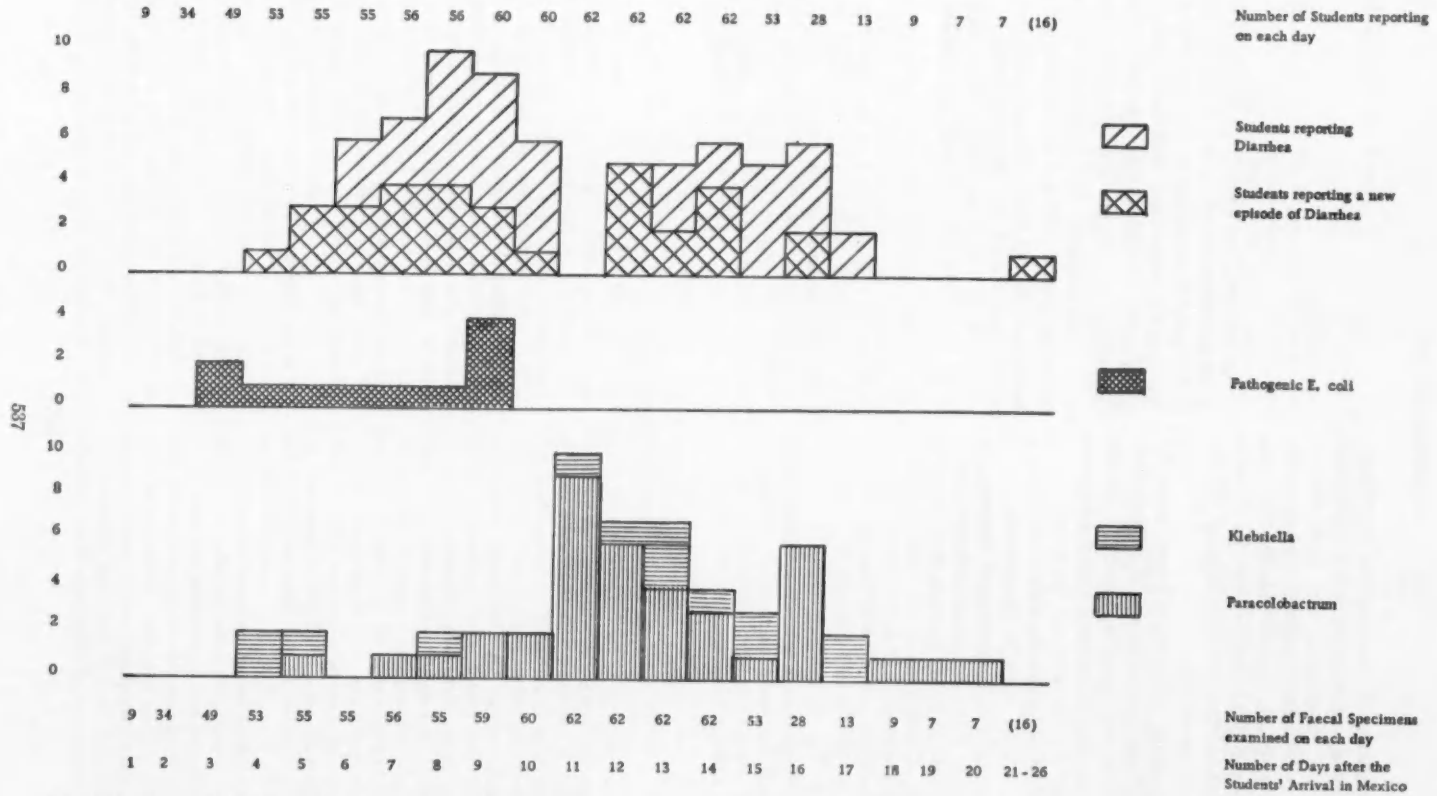
9 34 49 53 55 55 56 56 60 60 62 62 62 62 53 28 13 9 7 7 (16)
Number of Students reporting on each day
10
8
6
4
2
0
Students reporting Diarrhea
Students reporting a new episode of Diarrhea
4
2
0
Pathogenic E. coli
10
8
6
4
2
0
Klebsiella
Paracolobactrum
9 34 49 53 55 55 56 55 59 60 62 62 62 62 53 28 13 9 7 7 (16)
Number of Faecal Specimens examined on each day
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21-26
Number of Days after the Students' Arrival in Mexico

FIG. 1. Incidence of diarrhea and isolation of enteric bacteria among newly arrived students in Mexico, as shown by rearrangement of data reported by Varela *et al.* (1959), *Am. J. Trop. Med. & Hyg.*, **8**: 353–357. The recurrence of diarrhea after an interval of more than 1 day was considered as a new episode of diarrhea.

I am aware that the presentation of available data has merely shown a statistical relationship between two phenomena, the occurrence of diarrhea and isolated bacterial pathogens. It could be that the isolated bacteria are only concomitant with the causal factors, for instance viral infections. This, however, remains for the present mere speculation.

The available evidence indicates that the isolated enteric pathogens are of an infectious nature and as such may be responsible for the clinical syndrome of the diarrhea among travelers to Mexico.

I am, Sir, etc.

Dr. O. Kranendonk
Laboratory of Hygiene
University of Amsterdam
Mauritskade 57

New York, April 25, 1960

To the Editor.

Sir,

The analysis of our data by Dr. O. Kranendonk is astute and provocative, and I hope you will find it possible to publish it. The conclusions in the original paper may well have been too conservative. Further studies were made during the summer of 1959 and will be reported before long. Unfortunately, the technique was not similar to that of 1958 and comparisons are not feasible.

Sincerely yours,

B. H. Kean, M.D.
728 Park Avenue

BOOKS RECEIVED

Aedes aegypti (*L*); *The Yellow Fever Mosquito*, by Sir S. Rickard Christophers, C.I.E., O.B.E., F.R.S., I.M.S. (Ret'd). 739 + xii pages, ill. Cambridge, Cambridge University Press (32 E. 57th St., N. Y. 22, N. Y.), 1960. $14.50.

Antibiotics Annual 1959-1960 (*7th Annual Symposium on Antibiotics*). Henry Welch, Ph.D., and Felix Marti-Ibañez, M.D., editors. 1034 + xx pages, ill. New York, N. Y., Antibiotica, Inc., 1960. $15.00.

Biological Organisation: Cellular and Subcellular (*A Symposium*), C. H. Waddington, editor, 328 + xviii pages, ill. New York 22, N. Y., Pergamon Press, Inc., 1959. $12.50.

The Origin of Life on The Earth (*A Symposium*). Edited for the Academy of Sciences of U.S.S.R., by A. I. Oparin, A. G. Pasynskii, A. E. Braunshtein, and T. E. Pavlovskaya. English, French and German edition edited for International Union of Biochemistry by F. Clark and R.L.M. Synge. Vol. I, 691 + xv pages, ill. New York 22, N. Y., Pergamon Press, Inc., 1959. $15.00.

Laboratory Identification of Pathogenic Fungi Simplified, by Elizabeth L. Hazen, Ph.D., and Frank Curtis Reed, 2nd edition, 150 + xv pages, ill. Springfield, Ill., Charles C Thomas, 1960. $7.50.

A Short Synopsis of Human Protozoology and Helminthology, by L. R. S. Macfarlane, O.B.E., M.D., M.A., D.P.H., Brig'r. (Ill. by R. M. Leach, B.E.M.), 1st edition, 251 + vii pages, ill. Baltimore, The Williams and Wilkins Co., (Exclusive U. S. Agents), 1960. $7.50.

Surgery and Clinical Pathology in the Tropics, by Charles Bowesman, O.B.E., B.A., M.D., F.R.C.S.E., F.A.C.S., D.T.M. & H., (Ed.)., 1st edition, 1068 + viii pages, ill. Edinburgh and London, E. & S. Livingstone, Ltd. (The Williams and Wilkins Co., Baltimore, exclusive U. S. Agents), 1960. $22.50.

Fundamentals of Clinical Hematology, by Byrd S. Leavell, M.D., and Oscar A. Thorup, Jr., M.D., 1st edition, 503 + xi pages, ill. Philadelphia, W. B. Saunders Co., 1960. $10.00.

BOOK REVIEWS

Fundamentals of Clinical Hematology, by Byrd S. Leavell, M.D., and Oscar A. Thorup, Jr., M.D. 1st Ed., 503 + xi pp., ill. Philadelphia, W. B. Saunders Co., 1960. $10.00.

This textbook is designed primarily for the medical student "who is being introduced to hematology for the first time while he is in the midst of a crowded curriculum." It is also intended to be of use to busy physicians and to ancillary workers as an up-to-date review and reorientation in the field. It achieves both of these aims. The style is concise, direct and factual yet readable. The material is up-to-date.

The approach is based on "comprehensive but not encyclopedic" descriptions of normal morphology, biochemistry, physiology and function of various cell lines, and of normal erythropoiesis, leukopoiesis, hemostasis, etc. These lead into the anemias, polycythemia, coagulation and disorders of hemostasis, the leukemias and therapy of leukemia and malignant lymphoma, and into other diseases of lymphoid tissues and bone marrow.

The tabulated classification of anemias introducing a major section of the book is based primarily on *Mechanism of Production*: (I. Diminished Erythropoiesis: A. Nutritional, B. Bone Marrow failure; II Blood Loss, acute and chronic; III. Increased Hemolysis: A, Intracorpuscular, B. Extracorpuscular) with *Causes* and *Diseases or Clinical Syndromes* listed under coordinate headings. The discussions in general include brief historical outlines, pathogenesis, and current theory and practice in diagnosis and treatment though the book is not a practitioner's manual. Many of the clinical syndromes are pointed up by descriptions and discussions of actual cases; a useful feature which could well be extended in another edition.

Laboratory data as diagnostic aids are as sufficiently detailed as would be expected in a student's book on hematology, and there is an excellent closing chapter on hematological techniques, with annotations, comments and up-to-date references. Possibly the description of blood groups and antigens and transfusions, introducing this chapter, could more usefully be included as intro-

ductory material in the section on mechanisms of anemia or, in part, preceding the section on erythroblastosis fetalis. However the material given is excellent though the proponents of the Wiener theory of Rh antibodies and blood factors might like to see this system (recently adopted *pro tem* by the Committee on Medicolegal Problems of the American Medical Association and by the Section on Immunology of the American Academy of Forensic Sciences) discussed in more detail. The discussions of the anemias, and of disorders of hemostasis seem especially good.

Each chapter is extensively documented (over 350 references in the section on anemia), with a large proportion of recent references. It is unfortunate that costs of color plates, now prohibitively high, prevent the use of more color in books of this type. It seems that colored reproductions of color photographs are perhaps more realistic and less idealistic than lithographs or plates made from paintings. However, photographic reproductions also are costly. This difficulty is a general one and not concerned specifically with the volume under review.

The proof reading is excellent and the typography, format and manufacture of the book reflect credit on a publisher long experienced in this field.

M. Frobisher

A Handbook of Diseases of the Skin, by Henry O. Mackey, F.R.C.S.I., L.R.C.P.I., D.P.H. (University of Dublin), L.M., F.R.I.A.M. 6th edition, 263 pages, ill. Dublin, C. J. Fallon Ltd., 1959. 8/6.

"This book is intended primarily for students as a practical guide to the clinical study and treatment of diseases of the skin." With this objective as his goal the author has succeeded in compressing much useful information and many illustrative photographs into the confines of a volume small enough to be inviting.

To be useful, however, such a guide should contain only information which is correct by present standards and it should not lead into error those readers who are insufficiently experienced to recognize debatable teachings. One may question the validity of several statements in the text, especially that "the lesions of impetigo tend to spontaneous cure in about three weeks," that "Sarcoidosis is probably a peculiar form of hematogenous skin tuberculosis associated with visceral tuberculosis" and that in pityriasis rubra pilaris "the general tendency is to spontaneous cure." In discussing lupus erythematosus the author states that "Lupus vulgaris is simulated by a rare type called lupus vulgaris erythematoides," implying erroneously that this entity is a form of lupus erythematosus.

In recommending treatment the author goes counter to present trends when he advises that "lesions of lichen planus on the genitals are best treated by Grenz rays or Thorium X" and that for a seborrheic wart "on the face the radium plaque in contact gives the best cosmetic result."

The formulary gives many prescriptions that are useful in practice, but a formulary, like a cook-book, must be infallible to be safe for beginners. The use of both the apothecary and metric systems is a hazardous pitfall and there are errors. The formula for Pusey's calamine liniment for example, prescribes 4 times as much zinc oxide and 60 times as much oil of bergamot as was intended and the result might prove disastrous.

The illustrations are numerous and many of them occupy the full page. Unfortunately many are dark, some are cropped too closely and many are too indistinct to instruct the novice effectively in the actual appearance of the dermatoses they illustrate.

Marcus R. Caro

CORRECTION

In the article "An enriched, egg extract medium for recovering viable *Entamoeba histolytica* trophozoites from fecal suspensions kept at room temperature for four days" by Neva N. Gleason, Morris Goldman and R. K. Carver in the January 1960 issue, page 46, column 2, line 2, "2 mg Trypticase (BBL)" should read "2 grams Trypticase (BBL)."

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and arthropod-borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted articles which raise no question of scientific priority but for which immediate publication seems important will be inserted in the next available issue of the journal if the author or sponsoring institution will pay the cost, estimate of which may be obtained from the editor. The insertion of such articles does not affect publication of other manuscripts awaiting their turn, since the inserted articles constitute additional pages in the volume without additional cost to the subscriber.

Publication will be facilitated if the following suggestions are followed:

1. *Manuscripts* should be typewritten in *English* using *double spacing* on *one side* only of 8½ x 11 inch paper and submitted *in duplicate*. Double-spacing should be used throughout the manuscript including the list of references, footnotes, and quotations.

2. The author should supply, on a separate sheet, a short title not over 50 letters for use as a running head. Also on separate sheets type (a) the authors' names and affiliations; (b) tabular matter; (c) case histories; (d) quotations; (e) formulas and other subsidiary matter in the text; (f) footnotes; (g) references; (h) legends for illustrations; (i) displayed formulas occupying more than 1 line of type, even if using the same size type as the main text.

3. Legends should be separate and not attached to, or written on, the illustration copy.

4. Positions for tables and figures in the text may be indicated on the margin of the text pages.

5. All papers should end with a brief summary.

6. *Tables* should be typed on separate sheets and be serially numbered in Arabic numerals.

7. *Figures* and *photographs* should be protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be made with India ink and submitted on white (not cross-section) paper. Colored illustrations can be reproduced only at the expense of the author.

8. References should be cited by consecutive numbers in the text, and so numbered and listed in the list of references at the end of the article, without regard to alphabetical arrangement. References should be written as follows:

> 1. Reed, L. J., and Muench, H., 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.*, **27**: 493–497.

9. *Proprietary names* of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U. S. Pharmacopoeia, New and Nonofficial Remedies of the A. M. A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the summary.

10. **N. B. The address to which proofs are to be sent should be indicated at the top of the first page of the manuscript. Delay will result if proofs must be forwarded.**

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¶ Bacto-Latex *0.81 micron* is characterized by uniform particle size, batch reproducibility and biological inertness.

¶ An inert carrier for use in clinical and investigational tests including

RHEUMATOID ARTHRITIS
INFLAMMATORY DISEASES
TRICHINOSIS
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Literature available on request

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